# The Metabolic Basis of Inherited Disease

# The Metabolic Basis of Inherited Disease

EDITORS

**John B Stanbury, M D**
*Associate Clinical Professor of Medicine*
*Harvard Medical School*

**James B Wyngaarden, M D**
*Associate Professor of Medicine and Biochemistry*
*Duke University Medical Center*

**Donald S Fredrickson, M D**
*Member Senior Research Staff National Heart Institute*
*National Institutes of Health*

*The Blakiston Division*
McGRAW HILL BOOK COMPANY, INC
New York Toronto London

THE METABOLIC BASIS OF INHERITED DISEASE



Library of Congress Catalog Card Number 60-8845

60720

## Contributors

---


J A M AGER M B
Lecturer in Pathology St Thomas s Hospital Medical School London England

JAMES A BAIN Ph D
Professor and Chairman Department of Pharmacology Emory University School of Medicine Atlanta Georgia

FREDERIC C BARTTER M D
Chief Laboratory of Clinical Endocrinology National Heart Institute Bethesda Maryland Associate Professor of Medicine Georgetown University Medical School Associate Professor of Pediatrics Howard University Medical School Washington D C

ALEXANDER G BEARN M D
Associate Professor Rockefeller Institute Physician to the Hospital of the Rockefeller Institute

ERNEST BEUTLER M D
Chairman Department of Medicine City of Hope Duarte California Associate Professor of Medicine University of Southern California

ROSEMARY BIGGS Ph D
Scientific Officer Medical Research Council The Blood Coagulation Research Unit the Churchill Hospital Oxford England

MAURICE B BURG M D
Associate in Research Section of Kidney and Electrolyte Metabolism Laboratory of Kidney and Electrolyte Metabolism National Heart Institute Bethesda Maryland

CHARLES H BURNETT M D
Professor of Medicine and Chairman Department of Medicine School of Medicine University of North Carolina Chapel Hill North Carolina

GEORGE F CAHILL JR M D
Director Metabolic Unit Peter Bent Brigham Hospital Associate in Medicine Harvard Medical School Boston Massachusetts

JEROME W CONN M D
Professor of Internal Medicine Director of the Division of Endocrinology and Metabolism of the Metabolic Research Unit University of Michigan Medical School Ann Arbor Michigan

JOSEPH DANCIS M D
Associate Professor of Pediatrics New York University Bellevue Medical Center New York N Y

T DAVID ELDER M D
Research Fellow in Medicine, Duke University Medical Center Durham North Carolina

RICHARD A FIELD, M D
Assistant Physician Massachusetts General Hospital Investigator Howard Hughes Medical Institute Associate in Medicine Harvard Medical School Boston Massachusetts

THOMAS B FITZPATRICK M D Ph D
Chief of Dermatological Service Massachusetts General Hospital Edward Wigglesworth Professor of Dermatology Harvard Medical School Boston Massachusetts

DONALD S FREDRICKSON M D
Member Senior Research Staff Section on Metabolism Laboratory of Cellular Physiology and Metabolism National Heart Institute Bethesda Maryland Special Lecturer in Internal Medicine George Washington University School of Medicine Washington D C

LAMONT W GASTON M D
National Science Foundation Postdoctoral Fellow Research Fellow in Medicine Massachusetts General Hospital and Harvard Medical School Boston Massachusetts

PARK S GERALD M D
Assistant Physician and Assistant Hematologist Children s Hospital Medical Center Associate in Pediatrics Harvard Medical School Boston Massachusetts

ROBERT S GORDON JR M D
Member Senior Research Staff Section on Metabolism Laboratory of Cellular Physiology and Metabolism National Heart Institute Bethesda Maryland Clinical Instructor in Medicine George Washington University Medical School Washington D C

HOWARD H HIATT M D
Associate in Medical Research Beth Israel Hospital Assistant Professor of Medicine Harvard Medical School Boston Massachusetts

ALAN F HOFMANN M D
Clinical Associate Section on Metabolism Laboratory of Cellular Physiology and Metabolism National Heart Institute Bethesda Maryland

R RODNEY HOWELL M D
Research Fellow in Pediatrics and Medicine Duke University Medical Center Durham North Carolina

CHARLES M HUGULEY JR M D
Associate Professor Department of Medicine Emory University School of Medicine Atlanta Georgia

KURT J ISSELBACHER M D
Assistant Physician and Chief Gastrointestinal Unit Massachusetts General

Hospital Assistant Professor of Medicine Harvard Medical School Boston Massachusetts

JAMES H JANDL M D
Research Associate Thorndike Memorial Laboratory Boston City Hospital Assistant Professor of Medicine Harvard Medical School Boston Massachusetts

JOHN B JEPSON M A B Sc D Phil F R I C
Senior Lecturer in Biochemistry Courtauld Institute of Biochemistry Middlesex Hospital Medical School London England

W EUGENE KNOX M D
Assistant Professor of Biological Chemistry New England Deaconess Hospital and Harvard Medical School Boston Massachusetts

STEPHEN M KRANE M D
Assistant in Medicine Massachusetts General Hospital Fellow Helen Hay Whitney Foundation Instructor in Medicine Harvard Medical School Boston Massachusetts

BERT N La DU M D Ph D
Senior Investigator Section on Arthritis and Rheumatism Laboratory of Clinical Investigation National Institute of Arthritis and Metabolic Diseases Bethesda Maryland

ALEXANDER LEAF M D
Associate Physician Massachusetts General Hospital Investigator Howard Hughes Medical Institute Assistant Professor of Medicine Harvard Medical School Boston Massachusetts

HERMANN LEHMANN M D Ph D Sc D M R C P F R I C
Associate Chemical Pathologist St Bartholomew s Hospital London England Senior Lecturer in Chemical Pathology St Bartholomew s Hospital Medical College London England

MORTIMER LEVITZ Ph D
Associate Professor Department of Obstetrics and Gynecology New York Hospital Bellevue Medical Center New York N Y

JOSEPH S McGUIRE JR M D
Investigator National Institute of Arthritis and Metabolic Diseases Bethesda Maryland Present address Department of Dermatology Yale University School of Medicine New Haven Connecticut

JACK ORLOFF M D
Chief of the Section of Kidney and Electrolyte Metabolism Laboratory of Kidney and Electrolyte Metabolism National Heart Institute Bethesda Maryland Associate in Medicine George Washington University Medical School Washington D C

RALPH E PETERSON M D
Associate Attending Physician The New York Hospital Associate Professor of Medicine Cornell Medical College New York N Y

ALBERT E RENOLD M D
Director Baker Clinic Research Laboratory New England Deaconess Hospital Assistant Professor of Medicine Harvard Medical School Boston Massachusetts

RUDI SCHMID M D, Ph D
Assistant Physician Thorndike Memorial Laboratory Boston City Hospital Assistant Professor of Medicine Harvard Medical School Boston Massachusetts

LLOYD H SMITH Jr M D
Assistant Physician Massachusetts General Hospital Associate in Medicine Harvard Medical School Boston Massachusetts

MARY JANE SPIRO Ph D
Research Fellow in Medicine Massachusetts General Hospital and Harvard Medical School Boston Massachusetts

JOHN B STANBURY M D
Associate Physician Massachusetts General Hospital Associate Clinical Professor of Medicine Harvard Medical School Boston Massachusetts

DAVID H P STREETEN M B D Phil M R C P
Associate Professor of Medicine State University of New York Upstate Medical Center Syracuse New York Investigator Howard Hughes Medical Institute Formerly Assistant Professor of Internal Medicine University of Michigan Medical School Ann Arbor Michigan

H ELDON SUTTON Ph D
Assistant Professor of Human Genetics Department of Human Genetics University of Michigan Medical School Ann Arbor Michigan

GORDON M TOMKINS M D Ph D
Chief Section on Metabolic Enzymes Laboratory of Biochemistry and Metabolism National Institute of Arthritis and Metabolic Diseases Bethesda Maryland

T FRANKLIN WILLIAMS M D
Assistant Professor of Medicine Department of Medicine University of North Carolina School of Medicine Chapel Hill North Carolina

ROBERT W WINTERS M D
Assistant Professor of Physiology Department of Physiology University of Pennsylvania School of Medicine Philadelphia Pennsylvania

JAMES B WYNGAARDEN M D
Associate Professor of Medicine and Biochemistry Duke University Medical Center Durham North Carolina

GRACE J YESLEY M S
Records Librarian Department of Human Genetics University of Michigan Medical School Ann Arbor Michigan

# Preface

The astonishing growth of human biochemical genetics during the past few years has created a need for a critical and comprehensive account of those heritable disorders of metabolism for which an appreciable body of knowledge now exists This book is an effort to meet this need by presenting the pertinent clinical biochemical and genetic information concerning those metabolic anomalies which have been grouped under Garrod s engaging term the inborn errors of metabolism Recent studies have clarified the nature of a number of these diseases new disclosures have brought others within the group and indeed new syndromes have been uncovered

In order to secure authoritative presentations the editors enlisted the collaboration of investigators actively engaged in the intensive study of specific heritable diseases Each contributor was given ample freedom to organize his own material as he might see fit within the broad framework of a general format He was encouraged to include sufficient normal biochemical and metabolic material in the relevant fields in order to provide a firm foundation for a detailed exposition of the nature of the defect at the heart of the disease or condition under scrutiny He was also urged to indicate areas where further work is needed and to hazard some speculations from existing data In this manner we attempted to achieve a stimulating and contemporary interpretation of each disease against a background of normal human intermediary metabolism and physiology

An entirely satisfactory solution to the problem of the selection of topics and organization was not possible and many arbitrary decisions had to be made For example we omitted discussions of several diseases which might logically have been included such as the heritable disorders of connective tissue recently well reviewed by Victor A McKusick The problem of classification was resolved largely on a basis of convenience Most disorders were categorized according to superficial biochemical resemblance but some eluded this scheme and seemed best grouped by organ system of chief involvement This has been done with some misgiving and with full awareness that a genetically determined

disorder cannot logically be considered limited to one system of the body

Each chapter has been critically reviewed by each of the editors and a number have been reviewed by additional readers Among the latter we are particularly indebted to Drs William Beck John Bertles Roscoe Brady Charles Burnett, Evan Calkins Allen Crocker Bernard Davis Leslie J DeGroot Frank Engel Jordi Folch Pi Lamont W Gaston John Graham Mahlon Hoagland V M Ingram Richard Portwood Lloyd H Smith Jr Robert Stempfel Eberhard Trams and T Franklin Williams Many of the sections on genetics were reviewed by Dr H Eldon Sutton of the Department of Human Genetics of the Medical School of the University of Michigan The efforts at excellence put forth by all authors and their tolerant acceptance of suggested changes in their manuscripts have made the tasks of the editors pleasant indeed

Our secretaries Miss Elizabeth Callahan and Mrs Lenora Wells, have given us devoted and invaluable assistance Much of the illustrative material was skillfully drawn by Mrs Edith Tagrin of the Medical Art Department of the Massachusetts General Hospital The continued advice support and encouragement of Dr Walter Bauer Dr James Howard Means Dr Eugene A Stead Dr Philip Handler and Dr Robert Berliner have been of immeasurable help to the three of us

John B Stanbury M D
James B Wyngaarden M D
Donald S Fredrickson M D

## Contents

## PART IV

### Diseases Characterized by Evidence of Abnormal Lipid Metabolism

### (The Lipidoses)



## PART V

### Disorders of Steroid Metabolism



## PART VI

### Diseases of Purine and Pyrimidine Metabolism

## PART VII

### Diseases Primarily Manifest as Abnormalities in Metal Metabolism



## PART VIII

### Diseases of Porphyrin Metabolism



## PART IX

### Diseases Manifest Primarily in the Blood and the Blood forming Tissues



## PART X

### Diseases Manifest Primarily in Renal Tubular Transport

## PART XI

### Diseases Involving a Deficiency of Circulating Enzymes or Plasma Proteins

Part One

# Introduction

# Chapter 1

# Inherited Variation and Metabolic Abnormality

---

*The Editors*

Man is endowed with great genetic diversity While distinct stocks may be recognized by the anthropologist genetically homogeneous strains of man do not exist With the singular exception of monovular twins each member of the human race has a unique inheritance Inevitably individual variation is an obvious feature of man

Individual variation is partly hereditary and partly environmental The environmental factors which influence the expression of the hereditary traits are of great variety perhaps greater than those to which any other species is exposed And yet man s individuality varies only within relatively narrow confines His similarities include such chemical factors as composition of tissues and directions and rates of metabolic processes

Deviations from the norms of chemical constancy may occur for a variety of reasons These deviations may be simply extremes of distributions of traits Most deviations represent consequences of acquired dysfunction A significant number represent heritable disorders attributable to mutant genes These examples are important out of all proportion to their numerical incidence because of the lessons they teach of the nature of metabolic events and their dependence upon hereditary control

Not all mutations embarrass the host in dealing with his environment The benignity of some mutations has obscured their presence and possibly distorted our view of the constancy of metabolic processes Other mutations undoubtedly cause alterations in metabolism which are unrecognized because they are incompatible with viability Between these extremes lies a growing number of examples of deviant metabolism whose diverse clinical expressions belie a close kinship in origin These conditions all may root in a common soil represented by the etiologic formulation altered genetic information → abnormal structure of a given protein in the host → an effect upon the chemical activity of that protein

To be sure, for many of the conditions included in this volume this formulation is only a framework for speculation at present In a few instances however, information of such specificity concerning causal mechanism had been acquired that the term *molecular disease* has seemed an appropriate designation [1] The promise of continuing enlightenment in this area is bright, a fortunate state of affairs which is a tribute to a century of outstanding contributions in the converging fields of clinical medicine biochemistry and genetics

The subsequent chapters are concerned with the present state of advance in the biochemistry of inheritance and with many specific instances of genetically altered metabolism For the reader uncertain of the terrain which lies ahead, a brief pause for perspective is offered in the following few pages into both historical development and the general features of control of intermediary metabolism most applicable to the inherited diseases Other more extensive reviews devoted to this same purpose are available elsewhere [2-9]

## HISTORICAL CONSIDERATIONS

### CONCEPT OF INBORN ERRORS OF METABOLISM (GARROD)

Shortly before the turn of the present century Sir Archibald Garrod began his studies on alcaptonuria which were to culminate in his classic Croonian Lectures of 1908 [10] and in his monograph on *Inborn Errors of Metabolism*, which appeared in 1909 and again in 1923 [11] From his observations on alcaptonuria cystinuria albinism and pentosuria Garrod developed the concept that certain diseases of lifelong duration arise because an enzyme governing a single metabolic step is reduced in activity or missing altogether Garrod viewed the accumulation of homogentisic acid in alcaptonuria as evidence that this substance was a normal metabolite in the dissimulation of tyrosine and correctly attributed its accumulation to a failure of oxidation of homogentisic acid A half century later Garrod s hypothesis was proved by demonstration of the absence of homogentisic acid oxidase activity in the liver of a patient with alcaptonuria [12]

Similarly the failure of pigment formation in the skin in albinism the appearance of pentose in the urine in essential pentosuria and the excretion of large amounts of cystine in the urine in cystinuria were viewed by Garrod as the results of blocks in normal metabolic pathways He attributed the first instance to failure of melanin formation and the latter two to excretion of metabolites accumulating proximal to the block in metabolism His interpretation of the defect in cystinuria has required modification but the correctness of his remaining hypotheses has been supported by subsequent studies

The fundamental discovery of Mendel concerning the inheritance of plant characters [13] had only just been resurrected and given prominent consideration at the time when Garrod was most active The transmission of a single character as a governing agent for a single enzyme and accordingly, as one step in intermediary metabolism is a thesis which seems implicit in Garrod s writings, but it was never stated as such The term *gene* was not introduced by Johannsen [14] until 3 years after Garrod s Croonian Lectures and nearly 40 years more were to pass before Beadle [15] clearly formulated the 'one gene–one enzyme concept (see below)

Garrod s work was almost ignored by geneticists for a generation Indeed Beadle [8] was led to remark that an examination of the major textbooks of genetics in use in 1940 failed to reveal any mention of alcaptonuria Time has corrected this oversight, the contributions of Garrod now occupy a deservedly prominent place in genetics and have had a marked effect on conceptual thinking in clinical medicine as well

## THE CHEMICAL TRANSLATION OF GENETIC INFLUENCE

### *Plant Pigments*

Studies on the inheritance of flower colors initiated by a contemporary of Garrod probably were the first to demonstrate genetic influence at the molecular level These investigations begun by Wheldale [16–18] and extended by Scott Moncrieff [19] Lawrence [20] and many others come close to marking the beginnings of biochemical genetics Several types of changes at single loci in the structure of the principle water soluble plant pigments the anthocyanins are inheritable and these substitutions cause great differences in pigment color [21]

At present it is thought that a gene governing a given chemical step in the production of plant pigments exerts its influence by controlling the synthesis or activity of an enzyme specific for that step [22 23] The early intuition of Wheldale that inheritible differences in flower color correspond to specific differences in pigment molecules was clearly a forerunner of the concept that a single chemical reaction may be under the control of a pecific genetic locus

### *One Gene–One En yme*

The concept mentioned above attained clear definition in the one gene–one enzyme principle first clearly stated by Beadle [15] This formulation of such fundamental importance to understanding of the inherited disea es emerged gradually from studies of eye color in the fruit fly *Drosophila* begun 30 years ago by Beadle and Ephrussi and co workers [24 25] It received extensive support from the classic studies of

Beadle and Tatum on induced mutants of *Neurospora crassa* in which the acquisitions of single essential growth factors were traced to single chemical reactions each dependent upon a different enzyme [8 26]

The one gene–one enzyme concept which Beadle developed from these experiments has been well expressed by Tatum [9] as follows

1 All biochemical processes in all organisms are under genic control

2 These biochemical processes are resolvable into series of individual stepwise reactions

3 Each biochemical reaction is under the ultimate control of a different single gene

4 Mutation of a single gene results only in an alteration in the ability of the cell to carry out a single primary chemical reaction

The one gene–one enzyme concept has recently been expressed in more sophisticated terms [27] embodying modifications that have developed since its inception These include a realization that the one gene–one enzyme concept does not require a one enzyme–one gene corollary and the replacement of the ' gene by progressively smaller units of ultimate genetic control Significant evidence contrary to the general theory has not been introduced however and many subsequent studies have supported it

*Inherited Diseases as Errors in Protein Synthesis*

The manner in which genetic information may govern the activity of an enzyme through altering its structure has been brought into sharp focus in the last decade by a series of brilliant studies on inheritable differences in structure of a nonenzymatic protein the globin portion of the hemoglobin molecule This work initiated by Pauling and his colleagues [1] is comprehensively reviewed in Chaps 2 and 34 and need be only briefly summarized here

It was first observed by Pauling Itano Singer and Wells [1] that sickle-cell hemoglobin moves differently in an electrical field than does normal hemoglobin a finding which suggested to them that there might be a difference in amino acid composition between the globin moieties of normal and sickle cell hemoglobin to explain their apparent difference in charge This inference was subsequently borne out by the elegant studies of Ingram [cf 28] Employing a finger printing technique for peptide identification he demonstrated that the structural difference between the two hemoglobins lay in only one of the many peptide fragments produced by trypsin hydrolysis Further analysis indicated this difference to be the substitution of one residue of valine for one of glutamic acid Thus the disadvantageous physical properties of sickle-cell hemoglobin result from a change in only one of several hundred amino acid residue A similar single substitution has now also been found to account for the different properties of several other hemoglobins

These exciting studies with hemoglobin have made it easy to visualize

how changes in the activity of an enzyme may be introduced under genetic control, since doubtless that activity is dependent upon many physical properties of the protein molecule which in turn are likely to be radically affected by minor structural changes

## ENZYMES AND METABOLIC REACTIONS

A detailed inquiry into the characteristics of enzymes and of mechanisms of enzymatic action is beyond the scope of this book [29] But since many disorders to be considered involve enzymatic derangements a few considerations are in order

An enzyme is a protein which functions as a catalyst of a biologic reaction The enzyme may catalyze a group reaction such as the oxidation of all aldehydes by aldehyde oxidase or it may be absolutely specific to one reaction and one substrate The velocity of a given reaction is dependent upon many factors the two most important of which are the inherent properties of the enzyme itself and the concentrations of reactants

### *Enzyme Quantity and Activity*

In addition to factors which affect the functional activity of a given quantity of enzyme there are factors which affect the amount of enzyme Since experimentally it is almost always the *activity* rather than the *quantity* of enzyme that is measured information on factors that influence the actual amount of enzyme present is meager

Enzymes are either induced or constitutive Induction may be positive or negative i e enzyme formation may be stimulated or repressed by factors such as substrate or product (direct or distant) or by other substances which act as inducers suppressors or activators and may in turn be under specific genetic control

Most enzymes in man and higher animals are constitutive in character as far as is known Their formation is controlled by factors other than the immediate environment their synthesis proceeds without the superimposition of a rate control determined by substrate product or readily altered inducer

Some of the inherited metabolic disorders of man may represent defects in enzyme induction and may therefore potentially be reflections of mutations of modifying or activating genes rather than of the primary gene itself An example may be the Crigler Najjar syndrome of hyperbilirubinemia in which the glucuronyl transferase is underactive This enzyme is normally deficient at birth but increases in activity shortly thereafter Such an increase fails to occur in the syndrome mentioned the failure possibly represents a failure of the inductive mechanism rather than a true absence of enzyme potential

Since enzyme excess is the rule for most reactions it follows that in general a reduction in the amount of enzyme must be considerable before

any limitation of activity becomes apparent indeed the factor of safety may be so great that almost a total lack of the enzyme is required before absence of its function can be detected This explains why it is so often difficult to detect an abnormality in the subject heterozygous for an enzymatic defect It also suggests that these heterozygotes may be detected by loading of their limited amount of enzyme with excessive substrate a device frequently employed

*Qualitative Change in Enzyme*

The net effect of a qualitative change in an enzyme may be exactly the same as a change in amount of enzyme synthesized because it is only the activity of the enzyme that has relevance to the economy of the organism Severe damage or deletion of a gene may result in complete failure to elaborate the enzyme under its control A lesser genetic change may result in formation of an enzyme protein with abnormal structure Such an alteration might change the function of the enzyme only slightly, if at all but it is apparent from the example of the hemoglobins *[28]* that a seemingly trivial change such as the substitution of one amino acid in a lengthy chain may have a drastic effect on catalytic function Indeed a *Neurospora* mutant has already been found which contains a protein indistinguishable antigenically from the enzyme tryptophan synthetase but which is enzymatically inactive, presumably the result of just such a minor and yet critical alteration in structure *[30]*

A number of examples exist in man of missing enzyme activity but thus far an inactive enzyme protein responsible for one of the inherited metabolic diseases has not been isolated and compared with its normal counterpart for structural flaws This is a formidable task geared to the pace of advances in the techniques for demonstrating not only protein structure but the relation of structure to function and for further isolating and identifying proteins presently recognized only by their enzymatic activity These means will be obtained in good time however and it is reasonable to predict that soon an 'inborn error of metabolism of man or mold will be convincingly demonstrated to derive from a change in the responsible enzyme molecule not more complex than that which distinguishes hemoglobin S from the normal pigment

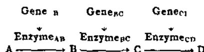
## CONSEQUENCES OF ALTERED PROTEIN SYNTHESIS

The consequences of a genetic change and an alteration in quality or quantity of a protein will depend upon the role normally subserved by that protein As indicated above one can generalize on this theme far beyond the role of proteins as enzymes to consider also their roles in structure and in transport phenomena Disabilities based solely on structural defects will not be considered in this volume even though numerous genetically determined disorders of this type are known A

number of disturbances of circulating proteins which do not function primarily as enzymes will be considered because of their instructive lessons Included in this group are the disorders of hemoglobin and of plasma proteins Also a number of disorders to be considered fall into a category suggestive of defects at the level of membrane transport Perhaps these conditions implicate malfunctions of carrier substances serving quasienzymatic roles However the majority of the disorders presently recognized and partially understood and included in this volume may be considered consequences of derangements of enzymatic function of the more conventional type or of defects of rate control of reaction sequences A brief inquiry into the nature of such derangements will provide an orientation for the chapters to follow [3 5-7]

*Blocked Metabolic Sequence*

Let us consider the model reaction sequence A ⟶ D and the controlling genes as follows



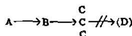

If gene$_{CD}$ is defective enzyme$_{CD}$ will be inactive and the reaction sequence will be blocked




A number of types of consequences may stem from such a block

1 *Lack of formation of product D or of some more remote product derived exclusively via D* A clinical disorder may result from failure to produce the immediate product D For example in von Gierke s disease the absence of glucose 6 phosphatase leads to an inability to make glucose from glucose 6 phosphate and hypoglycemia occurs on fasting Failure of specific steps in thyroxine and hydrocortisone synthesis leads to goitrous cretinism or the adrenogenital syndrome Alternatively a more remote product may not be formed as in albinism where melanin is lacking because of absence of tyrosinase an enzyme many steps proximate to melanin itself A secondary consequence of failure to form D may be a regulatory defect if D as an end product is involved in a feed back control mechanism (see below)

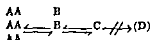
2 *Accumulation of C the immediate precursor of the blocked reaction*




This type of consequence may underlie various of the storage disorders such as the glycogen storage disease of liver or muscle due to absence of

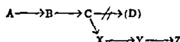
specific phosphorylases The substrate of the blocked reactions may accumulate in body fluids as bilirubin does when glucuronyl transferase is absent, or in a sense as uric acid does in species lacking uricase The substrate may accumulate sufficiently to lead to a notable increase in urinary excretion as in the case of homogentisic acid in alcaptonuria or of L-xylulose in pentosuria prototypes of blocked sequence correctly interpreted by Garrod

3 *Accumulation of A or B, remote precursors of the blocked reaction*



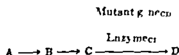

If reactions preceding the block are freely reversible or if the sequence can flow reversibly past unidirectional reactions because of the availability of a second or alternate route a precursor other than that just proximal to the block may accumulate In von Gierke s disease absence of glucose 6-phosphatase leads to gross accumulation of hepatic glycogen and elevated blood levels of lactic acid both several steps removed from glucose 6-phosphate the substrate of the blocked reaction

4 *Production of products normally of minor quantitative importance*




A block may result in an increased utilization of normally minor accessory pathways For example in phenylketonuria the block in phenylalanine hydroxylase results in an accumulation of phenylalanine and a gross over production of phenylketone products An analogous situation exists in maple sugar urine disease in which a block in decarboxylation of branched chain amino acids leads to accumulation and abnormal excretion of branched chain amino $\alpha$ keto and $\alpha$ hydroxy acids A block in glyoxylic acid metabolism may underlie the enhanced production of oxalic acid in primary hyperoxaluria In several of these disorders the abnormal product is found excessively in body fluids but certain disorders characterized by tissue storage lesions may eventually be shown also to be of this general type The possibility that accentuation of production of ordinarily lesser products involves enzyme induction secondary to accumulation of precursor $C$ may be kept in mind

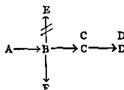
5 *Production of new product D*



If the gene alteration results in production of a functional enzyme differing in character from the normal enzyme perhaps a new product will result No certain examples of this type of defect exist among recognized hereditary syndromes but perhaps familial primary amyloidosis is a case in point

*Defects of Rate Control of a Metabolic Sequence*

In addition to those defects clearly attributable to blocks in known reaction sequences there are a number of disorders which appear to represent alterations of rates of normal reactions or reaction sequences Perhaps in some cases the apparent defect in rate control is a consequence of a partial or complete block in a side reaction of an early substrate leading secondarily to an increased utilization of that substrate in the reaction pathway to which attention has been drawn




For example a block in reaction $B \longrightarrow F$ might be recognized primarily as an augmentation of reaction sequence $B \longrightarrow C \longrightarrow D$ Over and above this type of situation in which a mistaken interpretation of the nature of the defect might be made there may be disorders representing more valid examples of primary derangements or variations of primitive rate control mechanisms By primitive is meant the type of control mechanism that might be operative in a single-celled organism or within single cells of a mammalian organism upon which neural or hormonal influences may be superimposed [31] A few disorders based on defects of hormonal control have been included in this volume when the disorder is clearly hereditary e g diabetes mellitus but in the present discussion they will not be further considered

It is probable that the regulation of metabolism operates always through variations of reaction velocities A number of types of primitive control mechanisms may be mentioned and at each level one may consider the potential consequences of a quantitative or qualitative change

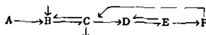
1 *Entrance of metabolite into the cell* Every membrane may be considered a diffusion barrier Substrates may cross this barrier with variable degrees of hindrance and the entrance of certain metabolites may be under enzymatic control The enzymatic control may be of several types including that of specific enzymes affecting membrane function such as permeases enzymes functioning as carriers in active transport phenomena or enzymes effecting removal of the substrate within the cell so as to

create or sustain a diffusion gradient A defect at this level has been suspected in Hartnup disease and may account for various of the disorders of renal tubular transport such as cystinuria and renal glycosuria

2 *Substrate concentration* As pointed out above in many reaction sequences the enzyme activities are sufficient to permit the sequence to operate at a rate well above its usual functioning level An example is urea synthesis in man which can increase considerably with protein loading In such cases rate control is vested in the concentration of the initial substrate of the chain It has been suggested that the increased purine synthesis of primary gout may represent a consequence of increased availability of glutamine due to some block in amino acid metabolism [7] or of phosphoribosyl pyrophosphate The levels of glutamine and PRPP may control the rate of synthesis of the key purine precursor phosphoribosylamine and therefore be controlling with respect to uric acid production

3 *Enzyme activity and the rate-controlling step* When a reaction sequence operates at capacity its velocity will be determined by the slow step which then becomes rate limiting Frequently the rate-limiting step is early in the chain but it need not be For example in conversion of glucose to lactate in intact ascites tumor cells [32] or in leukocytes [33] hexokinase is limiting and in glycolysis from endogenous glycogen in HeLa cells phosphorylase is limiting [34] However, in glycolysis in extracts of HeLa cells [34] the rate-limiting step may be phosphofructokinase or triose phosphate dehydrogenase depending on growth conditions of the HeLa cells The rate limitation may reside in either the enzyme quantity or cofactor availability of the key reaction

4 *Feed back control* In many biosynthetic pathways the rate of the reaction sequence is under control of an end product [35–37] In all known examples the control operates at the first unidirectional (irreversible) reaction concerned uniquely with synthesis of the product (*C* ⟶ *D* in sequence *A* ⟶ *F*)




Feed back control is of two type In the first the activity of the critical enzyme is regulated by the end product by a competitive type of inhibition the product and substrate compete for a binding site on the enzyme creating a chemostatic control designed to maintain constancy of supply of the product In the second the rate of synthesis of the critical enzyme is controlled by the product, which usually acts to inhibit enzyme synthesis but may act to augment it Thus this type of control may be repressive or inductive The first type permits rapid adjustments of velocity of a metabolic sequence by environmental factors the second

a slower regulation Mutational phenomena may lead to alterations of several theoretical types Enzyme$_{CD}$ might function normally in conversion of $C \longrightarrow D$ but might no longer be inhibited by $F$ or might be inhibited abnormally by some other product the biosynthetic capacity for making enzyme$_{CD}$ might be essentially normal but the stimulatory or repressive effect of regulatory metabolites might be changed or the biosynthesis of enzyme$_{CD}$ might be abnormal because of excessive response positive or negative to normal regulatory metabolites Possible implications of feed back control mechanisms are suggested in the defects leading to gross overproduction of orotic acid in orotic aciduria and of uric acid in primary gout

5 *Cytoarchitecture and compartmentation* It is becoming increasingly clear that cellular function is much more than the aggregate of isolated reactions even allowing for their complex interactions and elaborate interlockings [38] The structure of the cell may be controlling For example the characteristics of lactic dehydrogenase are quite different in solution and in contact with microsomes [39] Also ATP in mitochondria may not be freely available for use by soluble enzymes [34] Thus structural alterations within cell particles or of intracellular membranes may have important influences upon reaction velocities In congenital spherocytosis the de Toni Fanconi syndrome familial periodic paralysis and nephrogenic diabetes insipidus these considerations have been raised they reemphasize the critical role that cytoarchitecture may play in the orderly behavior of metabolic processes In a group of disorders suggesting end-organ failure such as nephrogenic diabetes insipidus pseudohypoparathyroidism and vitamin D-resistant rickets the possibility of a missing or abnormal binding protein necessary for local fixation of the hormone or vitamin must be considered

*Pleiotropism and Phenocopy*

There remain a number of situations in which a single genetic lesion gives rise to a diversity of phenotypic expressions Four mechanistic explanations for pleiotropism may be given

1 The mutation leads to failure of synthesis of an enzyme or other protein which acts as a catalyst inducer or suppressor of synthesis of one or more additional enzymes

2 The genetic defect gives rise to an abnormal material which in turn affects nonspecifically many functions and structures Sickle-cell hemoglobin is such a material

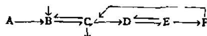
3 The defect gives rise to excessive production of a material which acts with some specificity to inhibit other apparently unrelated biosynthetic sequences For example phenylalanine and phenylketone products may act as inhibitors of other reactions (cf Chap 10) thus giving rise to defects of indole and of melanin production

create or sustain a diffusion gradient A defect at this level has been suspected in Hartnup disease and may account for various of the disorders of renal tubular transport such as cystinuria and renal glycosuria

2 *Substrate concentration* As pointed out above in many reaction sequences the enzyme activities are sufficient to permit the sequence to operate at a rate well above its usual functioning level An example is urea synthesis in man which can increase considerably with protein loading In such cases rate control is vested in the concentration of the initial substrate of the chain It has been suggested that the increased purine synthesis of primary gout may represent a consequence of increased availability of glutamine due to some block in amino acid metabolism [7] or of phosphoribosyl pyrophosphate The levels of glutamine and PRPP may control the rate of synthesis of the key purine precursor phosphoribosylamine and therefore be controlling with respect to uric acid production

3 *Enzyme activity and the rate-controlling step* When a reaction sequence operates at capacity its velocity will be determined by the slow step which then becomes rate limiting Frequently the rate limiting step is early in the chain but it need not be For example in conversion of glucose to lactate in intact ascites tumor cells [32] or in leukocytes [33] hexokinase is limiting and in glycolysis from endogenous glycogen in HeLa cells phosphorylase is limiting [34] However, in glycolysis in extracts of HeLa cells [34] the rate limiting step may be phosphofructokinase or triose phosphate dehydrogenase depending on growth conditions of the HeLa cells The rate limitation may reside in either the enzyme quantity or cofactor availability of the key reaction

4 *Feed-back control* In many biosynthetic pathways the rate of the reaction sequence is under control of an end product [35–37] In all known examples the control operates at the first unidirectional (irreversible) reaction concerned uniquely with synthesis of the product ($C \longrightarrow D$ in sequence $A \longrightarrow F$)




Feed back control is of two types In the first the activity of the critical enzyme is regulated by the end product by a competitive type of inhibition the product and substrate compete for a binding site on the enzyme creating a chemostatic control designed to maintain constancy of supply of the product In the second the rate of synthesis of the critical enzyme is controlled by the product which usually acts to inhibit enzyme synthesis but may act to augment it Thus this type of control may be repressive or inductive The first type permits rapid adjustments of velocity of a metabolic sequence by environmental factors the second

geal infections Alcaptonuria gout and even Gaucher s disease, may at times pose very little threat to a reasonably normal existence

**Severe to Lethal** On the other hand many of these conditions have severe consequences to the patient and may even be lethal Branched chain aminoaciduria and untreated phenylketonuria are associated with irreversible changes in the central nervous system The same is true of several of the lipid storage diseases which are often fatal in infancy The retarded development of congenital mutant cretinism may be irreversible Severe hepatic and neurologic disturbances accompany *Wilson s di ease* and many of the organ systems of the body are severely damaged in hemochromatosis The number of inherited metabolic disorders which are manifested only as abortion or stillbirth is of course unknown

*Age of Onset*

While the fundamental defects in these di orders must be congenitally present, they do not necessarily become clinically evident at an early age The relationship of the altered metabolic function to the phase of growth and development and the operation of other factors balancing the defect may affect greatly the age at which it is recognized Failure to thrive and retardation in development may be apparent almost from the day of birth in patients with branched chain aminoaciduria phenylketonuria galactosemia or metabolic cretinism Niemann Pick disease and Tay Sachs disease are commonly recognized within the first 6 months of life and the adrenogenital syndrome within the first several years

The majority of the hereditary metabolic diseases on the other hand become fully manifest only in the adult Several such as gout hemochromatosis or familial periodic paralysis rarely occur before the third decade Conversely some of these conditions improve as the patients become older Galactosemic patients if they can be helped through their early years with lactose free diets gradually develop the ability to metabolize galactose although their UDP galacto e transferase activity remains permanently mis ing For unknown reasons patients with adynamia episodica hereditaria and periodic paralysis also may cease having attacks in their later years

*Effects of Sex*

Some of the inherited metabolic di orders are sex linked such as hemophilia which appears almost exclusively in males and is transmitted by the female carrier Others are sex limited for reasons not always under stood For example there is at present no good explanation of why gout occurs so predominantly in males On the other hand hemochromatosis is undoubtedly far more common in males because females have in the men trual cycle a means of decreasing the iron available for tissue storage

4 The genetic defect may affect synthesis of peptide segments utilized in the eventual assembly of more than one protein A simultaneous lack of xanthine oxidase and a defect of renal transport of purines have been proposed in xanthinuria [40] The dual etiology of hyperuricemia in gout [41] raises the possibility of defects of both a biosynthetic enzyme and a renal tubular carrier involved in uric acid transport

The converse situation that of independent mutations and different metabolic abnormalities giving rise to disorders of apparent or even real similarity (phenocopy) is not difficult to comprehend Whether this is achieved experimentally by inducing a metabolic lesion in animals or whether it occurs spontaneously in man in the form of superficially identical disorders the principles to be considered are the same in any complex and interlocked metabolic sequence a number of defective loci may give rise to the same product deficiency or substrate accumulation or to abnormalities of the same protein

## EXPRESSION OF THE INHERITED METABOLIC DISORDERS

There is great variability in the expression of different inherited metabolic defects and even in the manifestation of one single defect in different individuals There are several points of reference from which such variability can be compared perhaps the simplest being the degree of incapacitation of the affected individual

### *Severity*

**Asymptomatic and without Consequence** Many of these disorders can be classified simply as metabolic variants' which are discovered accidentally Examples of such trivial but informative conditions which are difficult to classify as actual diseases are pentosuria most cases of fructosuria $\beta$ aminoisobutyric aciduria albumin B anomaly and the curious inherited differences in ability to taste the thiourea derivatives [42]

**Asymptomatic Except for Accidental Circumstances** Some of the inherited diseases become symptomatic only occasionally and then quite often because some accidental environmental circumstance has intervened Trauma is required for expression of many of the inherited deficiencies in blood clotting factors Several other diseases such as primaquine induced hemolytic anemia are revealed only by exposure to drugs and others such as acute hepatic porphyria may be gravely aggravated by them

**Mild to Moderate** The inherited diseases are sometimes troublesome and symptomatic but compatible with long life and productivity Patients with familial bilirubinemia are relatively asymptomatic unless they develop biliary stones Acatalasia seems benign in the absence of nasopharyn

## GENETICS AND EUGENICS

*Genetics and Incidence*

Since inherited metabolic diseases cannot be cured in a true sense the reduction of morbidity becomes mainly an exercise in eugenics Perhaps ultimately the prevention of harmful mutations will become a clearly defined public health discipline but it would be premature to devote space here to the present ragged outlines of this problem Of practical importance at the moment are means to decrease the clinical manifestations of existing mutations

Most of the diseases discussed in this volume have an autosomal recessive pattern of inheritance Care to exclude consanguineous marriages in tainted families will cause these diseases especially those with low gene frequency, such as phenylketonuria to become increasingly rare A lesser effect will be noted of course on the incidence of diseases with greater gene frequency such as drug induced hemolytic anemia

Di eases with an autosomal dominant mode of inheritance generally are limited in incidence only by any decline in fecundity of patients with the disease Here too the frequency of the disease in offspring and possibly a greater degree of severity in the presence of a double dose of the offending dominant gene are factors which may be influenced by the avoidance of doubly tainted matings

*Detection of Hetero ygotes*

The preceding paragraphs indicate the desirability of detection of the heterozygous genotype Considerable success has already been achieved Tests depend mainly upon detection of a failure of the enzyme to handle an abnormal load or even a normal load of substrate Thus in phenylketonuria it has been shown not only that there is reduced tolerance to an administered load of phenylalanine but that there is actually a slight elevation of the phenylalanine level in the blood of heterozygotes Heterozygotes of many other diseases have now been detected as will become apparent in subsequent chapters Improvement of detection techniques undoubtedly will continue but in many instances the detection of reduction in enzyme activity in the heterozygote may be quite impos ible except by direct analysis

*Eugenic Counseling*

Already biochemical methods are available for fairly accurate detection of heterozygotes in everal conditions As more tests become refined and available they will become important in marriage counseling [44] Even if the delicate equilibrium of the courtship → marriage equation fails to be influenced by unfavorable genetic information the lives of offspring

## THERAPEUTIC POSSIBILITIES

These diseases lend themselves unusually well to the ingenious employment of therapeutic maneuvers and often the results are at least as satisfactory as in the commoner diseases The type of approach varies with the nature of the defect and the means available to overcome it

*Limiting the Intake of a Precursor Which May Undergo Toxic Accumulation*

It is sometimes possible to withhold from the diet a substance whose metabolism is blocked and which is toxic or which gives rise to toxic metabolites through alternative metabolic pathways Considerable success has attended the treatment of phenylketonuric children with diets low in phenylalanine Phenylalanine itself is essential in only minute amounts providing tyrosine is in adequate supply and tyrosine being beyond the block is metabolized normally Galactosemic children improve remarkably on lactose free diets and the toxic form of fructosuria can be prevented by avoiding fructose

### *Supplying the Missing Metabolites*

When the metabolic block prevents formation of sufficient end product, the disease can sometimes be alleviated by supplying the needed metabolite The macrocytic anemia of the single case of orotic aciduria so far reported was relieved by feeding cytidylic and uridylic acids Much can be done to ameliorate metabolic cretinism if thyroxine is provided sufficiently early Administration of cortisone inhibits the stimulated pituitary of the adrenogenital syndrome, with gratifying relief of signs and symptoms

### *Environmental Manipulation*

The therapeutic resource in the group of patients with metabolic idiosyncrasy is obvious once the diagnosis has been made The avoidance of drugs known to induce hemolytic anemia in sensitive patients is effective and readily achieved Likewise the avoidance of cholinesterase inhibitors in sibships where hypersensitivity has been demonstrated [43] and of barbiturates in sibships in which there is a case of porphyria are to be strongly recommended

### *Depletion of Stored Substances*

It is sometimes possible to deplete the body of a storage substance with toxic properties Thus with British anti lewisite (BAL) or with penicillamine copper can be removed from the body with benefit to the patient with Wilson s disease Much can be done for hemochromatosis by frequent phlebotomy and classically uricosuric substances such as probenecid and salicylates are beneficial in gout by increasing the elimination of uric acid

affected with a condition like galactosemia or phenylketonuria can be greatly altered by early recognition and treatment made possible by such information

## BIBLIOGRAPHY


1 Pauling L Itano H A Singer S J and Wells I C Sickle cell anemia a molecular disease Science 110 543 1949
2 De Busk A G Metabolic aspects of chemical genetics Advances Enzymol 17, 393 1956
3 Dent C E Foreword Symposium on inborn errors of metabolism Am J Med 22 671 1957
4 Kalckar H M Some considerations regarding biochemical genetics in man Perspectives in Biol & Med 1 3 1957
5 Stetten D Jr A current view of metabolic errors Am J Med 26 660 1959
6 Snyder L H Fifty years of medical genetics Science 129 7 1959
7 Zollner N Inborn disorders of metabolism J Chron Dis 10 6 1959
8 Beadle G W Genes and chemical reactions in Neurospora Science 129 1715 1959
9 Tatum E L A case history in biological research Science 129 1711 1959
10 Garrod A E Inborn errors of metabolism (Croonian Lectures) Lancet 2 1 73 142 214 1908
11 Garrod A E *Inborn Errors of Metabolism* Oxford New York 1923
12 La Du B N Zannoni V A Laster L and Seegmiller J E The nature of the defect in tyrosine metabolism in alcaptonuria J Biol Chem 230 251 1958
13 Mendel G Versuche uber Pflanzenhybriden Engelman Leipzig 1901
14 Johannsen W The genotype conception of heredity Am Naturalist 45 129 1911
15 Beadle G W Biochemical genetics Chem Rev 37 15 1945
16 Wheldale M On the nature of anthocyanin Proc Cambridge Phil Soc 15 137 1909
17 Wheldale M Our present knowledge of the chemistry of the Mendelian factors for flower colour Part I J Genet 4 109 1914
18 Wheldale M Our present knowledge of the chemistry of the Mendelian factors for flower colour Part II J Genet 4 369 1915
19 Scott Moncrief R The genetics and biochemistry of flower color variation Ergebn Enzymforsch 8 277 1939
20 Lawrence W J C and Price J R The genetics and chemistry of flower color variation Biol Rev 15 35 1940
21 Wagner R P and Mitchell H K *Genetics and Metabolism* Wiley New York 1955
22 Jorgensen E C and Geissman T A The chemistry of flower pigments in *Antirrhinum majus* color genotypes III Relative anthocyanin and aurone concentrations Arch Biochem & Biophys 55 389 1955
23 Jorgensen E C and Geissman T A The chemistry of flower pigmentation in *Antirrhinum majus* II Glycosides of PPMMYY ppmmYY and PPMMYY color genotypes Arch Biochem & Biophys 54 72 1955
24 Beadle G W and Tatum E L Experimental control of developmental reactions Am Naturalist 75 107 1941
25 Ephrussi B Chemistry of eye color hormones of *Drosophila* Quart Rev Biol 17 327 1942
26 Beadle G W and Tatum E L Genetic control of biochemical reactions in *Neurospora* Proc Nat Acad Sci (U S) 27 499 1941
27 Horowitz N H and Leupold W Some recent studies bearing on the one gene one enzyme hypothesis Cold Spring Harbor Sympo ia Quant Biol 16 65 1951
28 Hunt J A and Ingram V M Allelomorphism and the chemical differences of the human haemoglobins A S and C Nature 184 1062 1958

when they recently examined human tissue cultures Chu and Giles [4] found that the 34 subjects which they studied all had 46 chromosomes Kodani [5 6] has reported the existence of three chromosome numbers—46 47, and 48—in gonadal tissues and has suggested that the chromosomes in excess of 46 are supernumerary The controversy over this once closed question has generated much interest A photograph of metaphase chromosomes from a human somatic cell (with 46 chromosomes) is shown in Fig 2 1

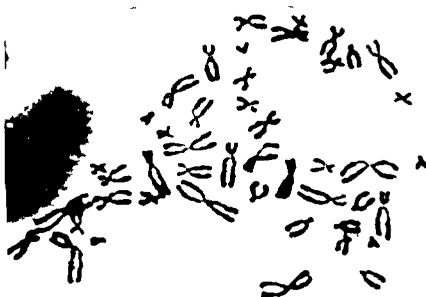


Fig 2 1 Human metaphase chromosomes prepared from a culture of connective tissue (*Courtesy of J Lejeune*)

Two recent findings illustrate the importance which cytologic studies of chromosomal status (karyotype) may have in the recognition and understanding of certain diseases In some persons with Klinefelter s syndrome an extra chromosome has been found giving a total of 47 [7 8] This extra chromosome resembles an X chromosome morphologically and these persons are presumed to have two X s *and* a Y chromosome The low frequency of X linked red green colorblindness (a recessive trait) in Klinefelter s syndrome is consistent with the presence of two X chromosomes [9] Analysis of the karyotype of mongolian idiots has also revealed the presence of an extra chromosome [10 11] In this case the supernumerary chromosome would presumably be an autosome

Two general mechanisms of chromosomal replication and division are known In *mitosis* the duplication of somatic cells each chromosome

# Chapter 2

# Human Heredity and Its Biochemical Bases*

*H Eldon Sutton*

## CYTOLOGIC BASIS OF INHERITANCE

The cytologic experiments of the last part of the nineteenth century provided the basis for the proposition that chromosomes are the structures responsible for the transmission of inherited traits These elongated nuclear bodies are governed in their replication by strict rules which ensure that each daughter cell receives the same complement of chromosomes as the parent cell possessed Although the chromosomal theory of inheritance has been amplified in many details its general principles have remained unchanged

In sexually reproducing higher organisms each somatic nucleus usually possesses a double *(diploid)* set of *chromosomes the corresponding* members of which are said to be homologous The germ cells *(gametes)* possess only a single *(haploid)* set Cells in certain tissues such as liver may occasionally possess higher multiples of the haploid number and are said to be *polyploid* Homologous chromosomes possess similar sets of genes normally arranged in identical linear fashion along the chromosomes An exception in many animals is the pair of chromosomes which determines sex Two unequal chromosomes are recognized designated X and Y In the human species females have two X chromosomes and males have an X and a Y chromosome Chromosomes other than the sex chromosomes are referred to as *autosomes*

The number of chromosomes varies widely among different species but is relatively constant within a species In man the number was reported by Painter [*1*] to be 48 (24 pairs) Although this number was confirmed by many observers Tjio and his associates [*2 3*] found only 46 chromosomes

This work was supported in part by Research Grant A 830 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health Public Health Service

These haploid cells are the germ cells (gametes) i e spermatozoa or ova A diploid zygote results from the fusion of a spermatozoon and an ovum

Although the distribution of chromosomes into daughter cells is random, the genes which are together on the same chromosome must obviously be transmitted as a group An exception to this occurs in the case of crossing over referred to above The genes which are on the same chromosome are said to be *linked* and form a *linkage* group The number of linkage groups would correspond to the haploid number of chromosomes in an organism Since the genes are linearly arranged on the chromosomes the frequency

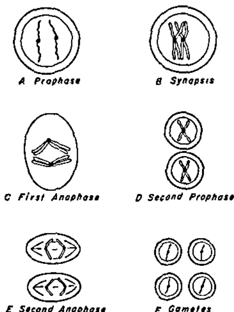



Fig 2-3 Meiosis A single pair of chromosomes is shown A Prophase B synapsis C first anaphase D second prophase E second anaphase F gametes

with which crossing over occurs between any two genes is a measure of their distance apart These distances are additive and are expressed as the per cent of *recombinants* (exchanged marker genes) found in the offspring of a mating involving two such recognizable genes Genetic distance does not show a 1 1 correspondence with physical distance although the two are related

For most organisms which have been studied extensively linkage maps can be constructed showing the position of each observed gene along a one dimensional scale In the case of man virtually all the known genes which can be assigned to a linkage group occur on the X chromosome There are over 40 such genes including those causing red green colorblindness and classical hemophilia Various mutant genes have also been reported to

reproduces itself and then splits lengthwise the two daughter chromosomes migrating to opposite ends of the cell which subsequently divides This process is illustrated in Fig 2 2 The important point to note in mitosis is that each daughter cell receives a full complement of chromosomes exactly like those of the parent cell from which it was derived Microscopic similarity of course is no proof of identity at the molecular level but there is evidence which suggests that the identity does extend to the molecular level

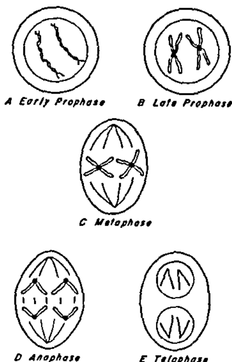



Fig 2 2 Mitosis Only a single pair of chromosomes is shown A Early prophase B late prophase C metaphase D anaphase E telophase

*Meiosis* the formation of germ cells is analogous to mitosis as shown in Fig 2 3 but there are some important differences Prior to separation of the daughter chromosomes (*chromatids*) the homologous chromosomes pair (*synapse*) lengthwise During this synapse bridges (*chiasmata*) are sometimes formed frequently resulting in a reciprocal exchange (*crossing over*) of homologous parts of the chromosomes This is a most important process in that it provides the species with new combinations of genes some of which may have selective advantage Following synapsis the homologous pairs separate and migrate to opposite ends of the cell which then divides Without further replication these cells again divide producing four daughter cells each with a haploid number of chromosomes

These as well as other criticisms have been answered by very highly purified transforming principles prepared more recently For example Hotchkiss [21] has obtained an active transforming preparation for pneumococcus which contains less than 0 02 per cent protein In addition crystalline pancreatic DNase in extremely low concentrations ($10^{-4}$ mg per ml) rapidly destroys the ability of an extract to tran form [22] It is difficult to avoid the conclusion that the active part of the transforming principle is indeed DNA

Evidence has also been obtained from studies of bacteriophage infection that DNA carries the genetic information This work is embodied in the classic paper by Hershey and Chase [23] It had been established that infective phage particles consisted of two substances—DNA and protein These workers grew the phage particles on $P^{32}$ labeled bacteria in order to label the phage nucleic acid and on $S^{35}$ to label the phage protein In a series of ingenious experiments they demonstrated that the phage particles consist of a core of DNA surrounded by a protein coat When the phage particles adsorb onto a sensitive bacterium the DNA is 'injected' into the bacterial cell the protein coat remaining outside If a strong shearing force is applied to the phage bacterium combination the protein coats (minus their DNA) can be recovered and shown to retain all the antigenic properties associated with the intact phage Removal of these protein coats from the bacteria after the DNA has entered the cells does not alter the course of the infection of the bacteria by the phage In these experiments a small amount of protein—about 3 per cent of the injected mass—actually does enter the cell This very small amount however is not thought to play a major role in the transfer of genetic information

An apparent exception to the rule of DNA as the genetic material is found in the plant viruses The extensively studied tobacco mosaic virus for example consists of a long core of RNA surrounded by a cylinder of protein By careful treatment of the tobacco mosaic virus Fraenkel Conrat et al [24] have succeeded in separating the RNA from the protein and recombining it with other strains of tobacco virus The resultant combination is infective but produces progeny with characteristics of the strain from which the RNA was obtained rather than the strain from which the protein was obtained Gierer and Schramm [25] have treated tobacco mosaic virus to obtain RNA preparations which retain part of the infectivity of the intact virus In these plant viruses the RNA appears to take over the role occupied by DNA in other organisms

## STRUCTURE OF DNA

The building blocks which make up the nucleic acids have been recognized for many year but the manner in which they are put together has been a most difficult puzzle to solve because of the immense size of the intact nucleic acid molecules (molecular weights greater than ten million)

occur on the Y chromosome, but as discussed later the existence of any gene on the Y chromosome has been questioned [12] The only linkages of autosomal genes which are generally accepted are between the Rh factors and elliptocytosis [13, 14] the ABO groups and nail patella syndrome [15] and the Lutheran group and secretor type [16 17] Evidence strongly suggesting linkage between other factors has been published An important fact to note is that two linked genes will show a correlation within single pedigrees but not within the population as a whole Morton has suggested the existence of two genes for elliptocytosis since this trait was linked to the Rh blood groups in only four of the seven large pedigrees which he studied [14 18]

## CHEMICAL STRUCTURE OF GENETIC MATERIAL

### DNA AND GENETIC INFORMATION

Chemical studies of nuclei both by histologic techniques and by assay of isolated nuclei have consistently revealed the presence of three major organic materials These are ribonucleic acid (RNA) deoxyribonucleic acid (DNA) and protein including a large amount of basic protein The chromosomes consist primarily of DNA and protein which are presumably combined as nucleoprotein

It was long assumed that the protein moiety was the carrier of the vast amount of genetic information necessary ultimately to specify the structure of all the protein synthesized in a living cell As experimental methods were improved it became obvious that the DNA should also be considered as a possibility

The first critical experiments to demonstrate that DNA can in fact transmit genetic information were carried out by Avery, MacLeod and McCarty [19] As early as 1928 Griffith [20] reported that killed pneumococci of one capsular type when injected into mice simultaneously with live pneumococci of another capsular type could lead to the recovery of live pneumococci whose capsule type matched that of the killed cells Avery and his coworkers extended these results by the preparation of extracts which had the transforming potency of the original bacterial cells They concluded that this transforming principle was DNA for the following reasons (1) quantitative elemental analysis showed it to resemble DNA (2) chemical studies revealed the presence of DNA (3) with the physical means available only a single DNA like substance was detectable (4) there were no antigenically active substances present and (5) deoxyribonuclease was the only enzyme among those tested which destroyed the transforming activity To be sure the presence of very small amounts of non DNA material could not be excluded, and the DNase available to these workers was not known to be free of other enzymes hence the possibility still existed that the genetically important substance was not DNA

of more than one polynucleotide chain with some information on the main dimensions of the helix (3) the approximate 1 1 molar ratio of adenine to thymine and guanine to cytosine suggesting that these bases occur in pairs and (4) the probable joining of the bases by hydrogen bonds Watson and Crick were able to construct a model which conformed to these findings and which agreed very nearly with the x ray diffraction data obtained from studies of crystalline DNA Their model shown schematically in Fig 2-4 consists of a right handed double helix formed by two polynucleotide chains which are relationally coiled The ' backbones of *the chains the sugar phosphate structure* are on the outside of the helix and the bases are on the inside of the cylinder A particular merit of the Watson Crick model is the specific pairing by hydrogen bonds of adenine with thymine and guanine with cytosine

Watson and Crick recognized that this model had several properties which could be used to explain certain genetic events [29] First the DNA chain could store information by virtue of the choice of four nucleotides at each nucleotide position Second each individual strand could direct the replication of its reciprocal structure If we picture the helix uncoiling with separation of the strands then each strand could serve as a template upon which free nucleotides could condense in a specific order These free nucleotides could then polymerize yielding two separated but identical double helices An interesting point in this model is that the daughter helices have arisen from homologous templates which are reciprocal rather than identical

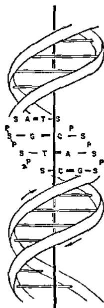
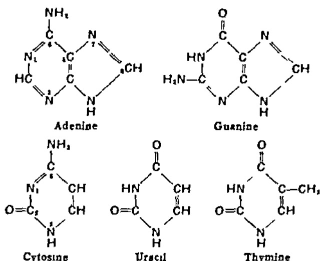


Fig 2-4 Diagram of double helix of DNA The double bonds indicate hydrogen bonding between specific base pairs (*By permission of J D Watson and F H C Crick* [71])

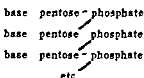
In only a few instances such as in bacteriophage is it perhaps proper to think of the chromosome as a naked piece of DNA The usual situation is to have the DNA in combination with proteins in particular the basic histones The exact nature of this combination is not known nor is the arrangement of DNA within the chromosomes of higher organisms Whether all the DNA in chromosomes is active genetically is yet to be ascertained

Of great interest has been the recent discovery of an unusual type of DNA in the bacteriophage $\phi$X 174 [*99a*] Physical studies indicate that this DNA consists of a single polynucleotide strand i e one half of a Watson Crick double helix The molecular weight of 2 million is considerably lower than that of ordinary DNA In many respects this single-strand DNA seems to be analogous to RNA which also is a single strand

The basic units in nucleic acids are the nucleotides consisting of a purine or pyrimidine base, a pentose (ribose or 2-deoxyribose) and phosphate. Five bases are commonly found in nucleic acids: the purines adenine and guanine, and the pyrimidines cytosine, uracil and thymine. Occasionally 5-methylcytosine may replace part of the cytosine, and in some bacteriophages 5-hydroxymethylcytosine replaces all the cytosine [26].




In the nucleotides the bases are attached by the N9 (purines) or N3 (pyrimidines) to the C1 hydroxyl of the pentose (furanose structure). The phosphate is attached to the C5 hydroxyl. In the polymerized form an additional bond is formed between the phosphate of one nucleotide and the C3 hydroxyl of the sugar in the next nucleotide, thus




There are several differences in chemical composition between the two types of nucleic acid. Ribose appears in RNA and 2-deoxyribose in DNA. An equally distinct difference occurs in the bases which comprise the two nucleic acids. Although adenine, guanine and cytosine are in both types, uracil occurs only in RNA and thymine only in DNA.

In addition to this chemical knowledge of nucleic acid structure, certain other facts had been established prior to the presentation in 1953 by Watson and Crick [27] of their molecular model of DNA. As reviewed by Wilkins [28], these were (1) the dimensions of the nucleotides (bond lengths, etc.), (2) the existence of a helical structure probably consisting

and if these segments do not overlap then by breaking and rejoining the two chromosomes at a point between the deletions one obtains one intact chromosome and one chromosome containing two deletions However if the deletions on the two chromosomes happen to overlap it is not possible to reconstruct a normal chromosome by this method This principle is valid also if one (or both) of the mutations is a point mutation rather than a deletion In the case of point mutations some normal progeny will result from mutations back to wild type and this number must be measured and subtracted from the total number of wild type progeny obtained from a cross of two different mutants By ascertaining which deletions and point mutations are able to yield normal recombinant offspring and by measuring the frequency of recombinant offspring produced by various



Fig 2 5 Preliminary locations of stable rII mutants Mutants producing no wild recombinants with each other are drawn in overlapping configuration Pairs which produce small amounts are placed near each other (*By permission of S Benzer [33]*)

pairs of mutants it is possible to establish a unique linear arrangement of the mutant loci In bacteriophage the formation of recombinants probably does not involve breakage and reunion of the parental chromosomes (or DNA molecules) Rather it seems to arise from a switch-over from one parental template to another on the part of the forming offspring molecule The consequences of this process are geometrically the same as for crossing over in higher organisms Figure 2 5 is a diagram of some of the deletions found at the rII locus of bacteriophage T4

The accurate mapping of closely linked point mutations requires the examination of many progeny but with bacteriophage this is a feasible undertaking The first step is to determine in what segment of the rII locus the point mutation is located This is most conveniently done by studying its ability to recombine with the known deletions After the mutation is located within a segment defined by a particular deletion its frequency

of approximately this molecular weight This bacteriophage may prove a most useful organism for the study of DNA (and RNA?) function

GENETIC FINE STRUCTURE

The ultimate structure of genetic material can be approached by genetic as well as chemical studies and both approaches have yielded rich rewards during the last few years The genetic approach is based on the fact that when two chromosomes (or DNA strands) come into close association during replication occasional progeny will arise in which crossing over has occurred Crossing over is observed in all higher plants and animals and in most lower organisms The frequency of recombination of any pair of genes on the same chromosome is proportional to the distance between them and in fact this distance is commonly expressed as the per cent of recombinants in the progeny of a mating of parents carrying the two gene markers (One hundred per cent = 100 map units A linkage group may be greater or less then 100 per cent although distances greater than 50 per cent must be obtained by addition of smaller distances ) If no recombination occurs then the two markers are considered to be alleles of the same gene or at least very closely linked In the latter case recombination might be so rare as to preclude observing it in organisms with few progeny and long generation times

Important genetic evidence on the internal structure of genes came with the discovery of pseudoalleles [*30*] Basically this phenomenon consists in the rare recovery of recombinant wild type progeny which are functionally normal from parents each of which possesses only mutant genes at the genetic locus under study In other words, two mutant genes are able to recombine and give a wild type offspring even though they are allelic

The solution to this problem has come only after an extensive series of studies by many workers The work of Benzer is perhaps the most revealing because of the power of genetic resolution possible with bacteriophage The fact that results with other organisms have yielded the same conclusions indicates the generality of these findings [*31*] Benzer [*32, 33*] studied a series of mutants in bacteriophage T4 which grows on *Escherichia coli* These r' mutants are recognized by the larger plaques which they form on sensitive host cells and they can be separated genetically into three groups depending on the section of the chromosome in which the mutations occur Benzer has examined the rII group in detail and has further separated these mutants into two types—those which never show a spontaneous reversion to wild type and those in which an occasional mutation to wild type occurs The former are best explained as *deletions* of genetic material while the latter can be considered as alterations at a single point (*point mutations*)

If by chance two chromosomes have each suffered deletion of a segment

functional groups then the nonmutant segments of each mutant complement each other in their activities and both mutants can grow If on the other hand both mutations are in the same functional group then there is no intact segment capable of carrying out this particular function and neither mutant will grow Such genetically identified functional units have been given the name *cistron* by Benzer because of their detection by the *cis trans* test of Lewis It is generally assumed that each cistron is responsible for the synthesis of a separate protein (or a polypeptide chain) In the case of the rII locus presumably two products are necessary to carry out whatever this function may be In applying the word cistron however it should be remembered that this term is defined with respect to a genetic test and not with respect to any chemical product

To take care of the two remaining definitions of a gene Benzer has coined the terms *muton* for the smallest mutable unit and *recon* for the smallest unit of recombination The present methods of genetic analysis permit one to set only an upper limit on the size of these units never theless this limit in both cases has approached the size of a single nucleotide pair

## REPLICATION OF DNA

A picture of the replication of the DNA double helix has already been presented in which the intertwined chains unwind each then serving as a template on which nucleotides condense and polymerize giving rise to a complementary strand There are still many problems to be solved in translating this attractive theory into fact among which is the unwinding problem This problem has not been made easier by the recent report that the true molecular weight of DNA may be of the order of 100 million rather than 10 million as previously thought [34a] In the case of a bacteriophage particle the DNA is much longer than the diameter of the cell in which it is replicating Intuitively one would reject the idea of such a tangled mass regularly unwinding and replicating However calculations by Levinthal and Crane [35] suggest that the energy required is not too great and that perhaps one s intuition is unreliable

The necessity of unwinding such a large structure is based on the assumption that DNA in vivo occurs in the same large pieces in which it is usually isolated and furthermore that these large pieces are not temporarily broken down during DNA replication Direct proof of this in most organisms is difficult but it has been possible to demonstrate that parental DNA does not become randomized during replication The same experiments demonstrate that parental DNA is divided equally between daughter cells Thus there is a mechanism of replication which involves a regular division of the DNA molecules into two equal parts

of recombination with other mutants within the same segment is ascertained. The results of the analysis of such a series of mutants by Benzer is shown in Fig 2 6. This series of mutants occurs within a deletion designated r164. Of 145 reverting mutants found within this region 123 occurred at the same point (called r131 after the first mutant found at this point) the remainder being distributed at 10 different points. This nonrandom distribution points out the very interesting fact that mutations are likely to occur at certain sensitive points rather than at random

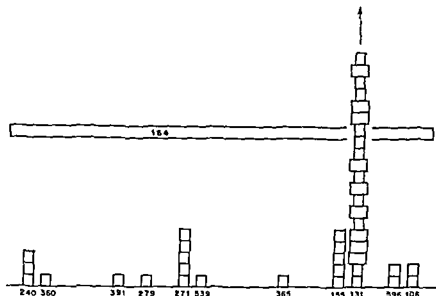



Fig 2 6 Grouping into species of mutants within the segment defined by the deletion r164. The arrow indicates 103 additional mutants of the r131 species. The different-size boxes of the r131 species indicate two distinct varieties based on the reversion rates. The number assigned to each location is that of the first mutant found at that position. (*By permission of S Benzer [33]*.)

Such a mutation profile may vary considerably with the mutagenic agent, some points being sensitive to one mutagen but not to another [34].

An analysis of genetic fine structure such as just described raises questions concerning the definition of a gene. Traditionally a gene has been defined in three ways—as a unit of function, as a unit of recombination and as a unit of mutation. In dealing with widely separated markers there is no experimental incompatibility among these definitions. In the rII locus of bacteriophage T4, however, there is an illustration of the contradictions which can exist. For example, in mapping the rII mutants Benzer discovered that they could be divided into two functionally independent groups designated *A* and *B* in Fig 2 5. This functional independence can be ascertained by infecting an appropriate strain of *E. coli* simultaneously with two rII mutants. If the two mutations are in different

labeled DNA In accord with the other experiments cited they found that a single replication reduced the label by one half forming hybrid molecules of half $N^{15}$ and half $N^{14}$ Heat denaturation of the hybrid DNA reduced the molecular weight by one half and at the same time permitted recovery of pure $N^{15}$ and pure $N^{14}$ subunits The most reasonable explanation is that denaturation caused the double helix to unwind, yielding a strand of $N^{15}$ parental DNA and a strand of $N^{14}$ daughter DNA Thus the original postulate of Watson and Crick [29] on DNA replication is substantiated

## GENE ACTION AND SPECIFIC PROTEINS

### RELATIONSHIPS OF NUCLEIC ACIDS TO PROTEIN SYNTHESIS

All known biochemical reactions in organisms depend ultimately on proteins for their catalysis Of first importance in the study of gene action is the manner in which proteins are synthesized with particular reference to the nature of protein specificity

The first suggestions that nucleic acids control the synthesis of proteins were made by Caspersson [38] and by Brachet [39] reasoning primarily from their cytologic studies By the use of such techniques as ultraviolet microspectrophotometry Feulgen staining and enzyme digestions it was demonstrated that the DNA occurs exclusively in the nucleus while RNA occurs both in the nucleus and in the cytoplasm Furthermore the localization of DNA in the chromosomes and its regular increase and distribution with cell division suggested DNA as an important part of the primary genetic system [40] RNA on the other hand varies among different cell types within an organism and is the nucleic acid present in the nucleolus If one compares the amount of RNA in different cells with the rate of protein synthesis by those cells a good correlation is obtained The silk gland of the silk worm has the highest RNA content known Liver and pancreas have a very high RNA content while heart muscle and kidney cells have a low RNA content The rates of protein synthesis in these last are low although their metabolic activity is high This association suggests a relationship between RNA level and protein synthesis [41]

Since the work of Caspersson and of Brachet many experiments have been performed with the purpose of demonstrating the association of nucleic acids and protein synthesis Most of the earlier conclusions were based entirely on circumstantial evidence With the preparation in recent years of cell free systems able to incorporate amino acids into proteins the study of protein synthesis has been put on a firm experimental basis which is yielding results truly valid at the molecular level These will be reviewed briefly although it is apparent that there are still tremendous gaps in our knowledge

One of the first experiments to show that DNA replicates as a unit was conducted by Levinthal [36] who grew T2 bacteriophage on E coli which were heavily labeled with $P^{32}$ The intensity of labeling of DNA in the phage was sufficient for individual molecules to be studied with the autoradiographic technique If the original labeled phage were ruptured by osmotic shock so that the DNA was released into solution, approximately 40 per cent of the total label was found to remain intact in large pieces of DNA (molecular weight about 45 million) while the remaining 60 per cent was dispersed as small molecules If the phage were allowed to replicate in unlabeled bacteria for one or two generations the labeling was found still to be associated with a few intensely labeled particles However, each labeled particle now contained only 20 per cent of the original label

A recent study by Taylor et al [37] using labeled thymidine with *Vicia faba* (broad bean) is of interest because it shows the same results *in this higher plant Thymidine is incorporated only into DNA and* generally only during DNA synthesis Taylor exposed dividing root cells to the labeled thymidine for 8 hr (one third of a division cycle) after which they were transferred to colchicine for varying periods of time Colchicine inhibits cell division without inhibiting chromosome replication Autoradiographic studies indicated that those cells in which the chromosomes had replicated during exposure to thymidine possessed both sets of daughter chromosomes equally labeled After an additional replication only one of each pair of daughter chromosomes was labeled If the cells were allowed to replicate still another time half of the still attached daughter chromosome pairs were unlabeled while half showed a single daughter chromosome labeled

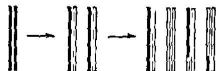


Fig 2-7 Replication of a single chromosome showing conservation of the original DNA within a half-chromosome strand Reciprocal exchange of homologous chromatid segments has occurred at the far right

These two studies provide conclusive evidence that whatever the precise mechanism of DNA replication it is conservative in the sense that half of each DNA complement is transmitted intact generation after generation and at no time during the life of a cell do the DNA constituents become randomly dispersed in the nucleus This is illustrated in Fig 2 7 These studies do not however tell whether the ' indivisible unit is a single polynucleotide strand a double helix or some more complex structure

Meselson and Stahl [37a] have obtained evidence that the indivisible unit of *E coli* DNA is most probably a single polynucleotide strand In experiments somewhat analogous to Taylor s they used $N^{15}$ to label DNA and ultracentrifugal techniques to differentiate labeled from un

appears in the nondialyzable deoxycholate soluble portion of the microsomes Since the RNA of the microsomes is almost exclusively in the small particles the soluble material would appear to be completed protein

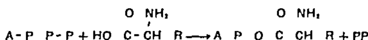
The above studies on the mechanism of amino acid incorporation have been done primarily with liver microsome preparations It has recently been demonstrated that labeled amino acids can be incorporated also into mitochondria and that in muscle tissue this may be the predominant site of incorporation [58] Although mitochondria have not been studied extensively it may be assumed that similar mechanisms of protein synthesis occur in both types of organelles since two equally efficient mechanisms are not likely to have arisen and persisted in the evolutionary contest for survival

As will be discussed later the inherited specificity of a protein is a function of the amino acid sequences of the constituent polypeptide chains and each sequence is rigorously specified The mechanism for arranging the amino acids into the proper order is still to be studied experimentally but Spiegelman has presented an impressive argument in favor of a template mechanism rather than a stepwise enzymatic mechanism [59] In order for a polyenzyme system to build up a polypeptide chain one amino acid at a time it would be necessary that each enzyme have built into it the information of the arrangement of most of the amino acids already incorporated so that it would act only at the proper moment This means essentially that each peptide bond of each protein would require a different enzyme The number of enzymes required would indeed be staggering particularly if one also considers the number of enzymes required to make the protein synthesizing enzymes

The alternate template mechanism assumes that the amino acids condense onto specific sites of some large structure where they are then joined together by peptide bond formation The formation of the peptide bonds could be catalyzed enzymatically but the enzymes would not be highly specific and would not carry the genetic information Thus each specific protein or perhaps polypeptide chain would originate from a specific template which would be the intermediate between the DNA of the gene and its product the protein

An interpretation of the template mechanism in terms of the present chemical knowledge of protein synthesis is crude nevertheless certain analogies can be noted which are most encouraging From the previous paragraphs it is obvious that the leading candidate for the role of template is RNA Hoagland et al [60] have presented a model in which the microsomal RNA acts as the template for the condensation of the soluble RNA–amino acid complexes The specific arrangement of the complexes is determined by the complementary relationships of the soluble RNA and the large RNA template Once in position the RNA moieties of the soluble complexes polymerize to form a large RNA molecule with

The first step in the condensation of amino acids into proteins involves the activation of the amino acids. The formation of a peptide bond requires an external source of energy. Lipmann [42] suggested in 1941 that the reaction proceeds through the formation of amino acyl phosphates. In 1955 Hoagland [43, 44] discovered soluble activating enzymes which catalyze the reaction between an amino acid and adenosine triphosphate to yield the mixed anhydride of the amino acid and adenosine monophosphate with pyrophosphate as the other product




The amino acid adenylates are firmly attached to the enzyme and probably do not exist in the free state in biologic systems although they can be separated from the enzymes under special conditions [45, 46]. These enzymes are highly specific for a given amino acid and a different enzyme is apparently necessary for each amino acid. Although all tissues have not yielded a complete array of activating enzymes it has been suggested that this is a result of experimental difficulties. The enzymes necessary for the activation of the 20 common amino acids have all been detected in extract from pigeon pancreas [47].

The activated amino acids are next bound to soluble RNA [46, 48]. The enzymes which catalyze the transfer of the activated amino acids to RNA are apparently the same as the ones which catalyze the formation of amino acyl adenylates. The process is visualized as the transfer of the amino acyl moiety of the amino acyl adenylate from the activating enzyme directly to soluble RNA [49]. The acceptor sites on the RNA are each specific for an amino acid as indicated by the observation that saturation of the RNA with one amino acid does not affect the ability of the RNA to accept other amino acids [50]. The point of attachment of the amino acids to the RNA is the adenosine group which occurs in the terminal position of soluble RNA [51]. The amino acid forms a covalent ester bond with the 3′ (or 2′) hydroxyl of the ribose [52]. The terminal adenylate appears to be linked through its phosphoric acid group to the 3′ hydroxyl of cytidine [53].

The next steps in protein synthesis are more obscure, and only a few generalizations can be made. It was established several years ago that incorporation of labeled amino acids into liver proteins occurs most rapidly in the microsomes [54, 55]. Fragmentation of the microsomes by deoxycholate has revealed the presence of small nucleoprotein particles which appear to be the actual centers for incorporation of amino acids [56]. The reactions leading to the incorporation of soluble RNA–amino acid complexes into the particulate RNA are not known but appear to require guanosine triphosphate [57]. The activity of labeled amino acids reaches maximum values in these particles within a few minutes and gradually

affected in their mutants This may often be the case but many mutations are now recognized which result in a detectable altered protein product Perhaps the best known of these in lower organisms although not entirely understood on the chemical level is the series of mutants for tryptophan synthetase in *Neurospora crassa* This enzyme is responsible for catalyzing the condensation of indole with serine to form tryptophan and mutants lacking the enzyme require the addition of tryptophan to the culture medium Suskind Yanofsky and Bonner [*66*] prepared antibodies to tryptophan synthetase and were able to demonstrate in most of the mutants the presence of cross reacting material (CRM) which lacked enzyme activity One of the mutants did fail to produce CRM proving the specific association of the CRM with the tryptophan synthetase locus

Levinthal and Garen (personal communication) have examined a large number of *E coli* mutants which fail to produce the normal amount of alkaline phosphatase activity under induction conditions These mutants could be divided into two groups—those in which no enzyme activity could be detected and tho e in which diminished enzyme ac tivity was present The relative ease of isolation of the enzyme from this organism made direct examination of the protein possible These studies still in progress have shown that most of the mutants with diminished activity produce an essentially normal amount of a protein product chemically similar to the wild type alkaline phosphatase but with a lowered catalytic activity By way of contrast most of the mutants which showed no enzyme activity also produced no protein analogous to the alkaline phosphatase

A fundamental problem concerns the genetic control of protein differ ences At least a partial answer has been found in studies of the hemo globin mutations in man The functional properties of proteins are gen erally considered to result from the nature of the surface such as contour distribution of charges etc This in turn results at least in part from the manner in which the long polypeptide chains are folded One can imagine that the folding of a polypeptide chain is controlled either by some other surface acting as a template or by the kind and sequence of amino acids which con titute the polypeptide chain In the case of immune antibodies it has generally been assumed that the specific surfaces result from differ ences in folding of a nonspecific protein blank Lederberg [*66a*] has sug gested that the evidence is more compatible with the hypothesis that the specificity is due entirely to the primary structure (amino acid sequences) of the antibody molecules

In the ca e of the abnormal hemoglobins the specific differences are known to be inherited and have been hown to result from changes in the amino acid sequence A difficulty in demonstrating this arose from the failure to find reproducible differences in the total amino acid com

configurational changes which promote the formation of peptide bonds between adjacent amino acids This last step would destroy the covalent bond between the amino acids and the RNA and the completed polypeptide chain would be free to separate from the RNA This model is consistent with most of the observations on protein synthesis, particularly the close association with RNA synthesis

The acceptance of a template mechanism still leaves the question of how the genetic information of the DNA is transformed into the specific structure of RNA At the molecular level, various models have been proposed but these all assume structures of RNA which are considerably extrapolated from actual knowledge It is probable that some template mechanism is again involved but the details must await further experimental results

The site of the information transfer is undoubtedly the nucleus since DNA is not found in the cytoplasm This is further indicated by such experiments as those of Goldstein and Plaut *(61)* who inserted $P^{32}$ labeled nuclei of amebas into unlabeled cells After several hours there was a transfer of labeled RNA from the labeled nucleus into the unlabeled cytoplasm With unlabeled nuclei in the presence of labeled cytoplasmic RNA there did not seem to be any transfer into the nucleus These and other experiments have produced a picture of nucleic acid-protein relationships in which the genetic DNA directs the synthesis of template RNA the latter diffusing from the nucleus into the cytoplasm where it in turn directs the synthesis of protein Once in the cytoplasm the RNA at least in some cases can replicate independently of the nucleus *(62)* A most interesting demonstration of the independence of protein synthesis from direct participation by DNA is found in enucleated reticulocytes where synthesis of hemoglobin continues after the nucleus has been extruded from the cell *(63)*

The preceding discussion has dealt primarily with cytoplasmic synthesis of protein Some protein synthesis also occurs in the nucleus (for example see Allfrey et al *(64)*) and as yet the direct intervention of DNA cannot be ruled out In particular it would seem possible that synthesis of the basic proteins which are part of the chromosomal structure might be under the direct control of DNA The nuclear enzymes on the other hand are probably controlled by the large amount of RNA in the nucleus

## NATURE OF THE PROTEIN PRODUCTS

All inherited differences are expressed eventually in the function of some protein either through changes in the efficiency or specificity of that protein or in the quantity of the protein Markert and Owen *(65)* studying a series of tyrosinase mutants in *Glomerella* were unable to detect any enzymatically inactive material which cross reacted with tyrosinase antibodies suggesting that the quantity of enzyme was

for hemoglobin S

val his leu thr pro val glu lys

It is now apparent that the differences in the properties of these two hemoglobins including a difference in solubility which gives morbid consequences in the case of individuals homozygous for hemoglobin S can be traced to a substitution of a single valine residue for a glutamic acid in each half molecule of hemoglobin

In a similar manner hemoglobin C was analyzed and found to be altered only in this same peptide the sequence being

val his leu thr pro lys glu lys

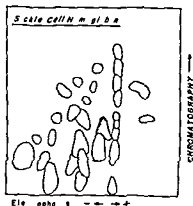
In this case lysine has been substituted for glutamic acid at the same position It should be noted that glutamic acid is capable of assuming a negative charge valine is neutral and lysine can assume a positive charge This corresponds to the differences in electrophoretic behavior observed with these hemoglobins

An interesting situation has developed with respect to another abnormal hemoglobin designated D [70] Samples from three unrelated individuals have been subjected to trypsin digestion each was found to differ from hemoglobin A in a single peptide but in each case it was a different peptide This raises the prospect that in some cases the hemoglobin designations are generic rather than specific This might be expected since not all changes involving a single amino acid residue in the midst of a large protein molecule would result in readily detectable variations in the behavior of the intact molecule This is particularly apparent if one considers the limited number of molecular properties available for study It raises the interesting thought that much undetected inherited variation may exist in normally functioning proteins and that many genetic mutations may occur which will be detectable only by the types of structural studies which have been applied to the hemoglobins

## GENE STRUCTURE VERSUS PROTEIN STRUCTURE

With the demonstration that a mutation can result in replacement of a single amino acid in a polypeptide chain it is tempting to relate the linear sequence of amino acids in proteins to the linear genetic fine structure of a chromosome In other words if one were to map genetically a series of point mutations such as Benzer has done in bacteriophage and compare the results with the relative positions of altered amino acids in the polypeptide chain which is the product of this genetic locus would the two series match? There is no answer as yet to this question One would expect however that the nucleotides necessary to specify a particular amino acid would be arranged in the same sequence along the DNA helix as are the amino acids in the polypeptide chain

position of the hemoglobins Fetal hemoglobin does have a clearly different amino acid composition from adult but it is produced by a different synthetic and presumably genetic mechanism [67] In an elegant study of the detailed chemical structure of hemoglobins Ingram [68, 69] has been able to demonstrate that there is a difference in amino acid composition between normal adult hemoglobin (Hb A) sickle-cell hemoglobin (Hb S) and the abnormal hemoglobins C and D (Chap 34) Crystallographic studies had previously shown that hemoglobin probably consists of two identical half molecules With a molecular weight of 66 700 there would be approximately three hundred amino acid residues in each half molecule Some thirty of these are the basic amino acids lysine and arginine Ingram digested a pure preparation of the particular hemoglobin with trypsin which hydrolyzes peptide bonds formed from the carboxyl group of lysine or arginine The resulting mixture of small peptides consisted on the average of 10 amino acids per peptide This mixture was subjected to electrophoresis on paper and then to chromatography at right angles to the electrophoresis The results for hemoglobins A and S are shown in Fig 2 8 It can be seen that the only difference between these hemoglobins involves a single peptide All other peptides were indistinguishable and presumably identical



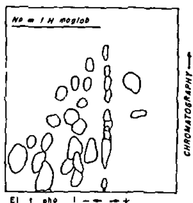




Fig 2 8 Polypeptide fingerprints of normal and sickle cell hemoglobins The shaded spots are those belonging to the peptide in which the amino acid difference exists (*By permission of V M Ingram [68]*)

This interesting peptide was isolated from the two hemoglobins and hydrolyzed to yield the individual amino acids In both cases, these were found to be histidine valine leucine threonine proline glutamic acid and lysine There was a difference however in that hemoglobin A contained more glutamic acid than hemoglobin S and conversely hemoglobin S contained more valine By partial acid hydrolysis a mixture of identifiable smaller peptides was obtained from which a unique sequence could be constructed For hemoglobin A this sequence proved to be

val his leu thr pro glu glu lys

ozygote It is important to note that in this case a single locus controls the specificity of a whole series of proteins and furthermore that the heterozygous individuals produce a series of proteins (hybrid substances) differing from either homozygote The explanation of this unusual system has yet to come The interaction of the alleles in the heterozygote—difficult to visualize at the gene level—suggests that a series of polymers may be involved Thus each allele may control the production of a single monomer, with subsequent reactions independent of this locus producing varying degrees of polymerization Consistent with this hypothesis is the recent observation of Smithies [81a] that treatment of haptoglobins with sulfhydryl reagents and urea produces protein subunits which correspond more closely to the genes present and may represent the monomeric forms This hypothesis does not explain all properties of the system

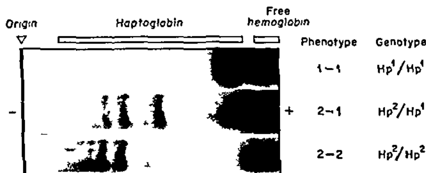



Fig 2 9 Plasma electrophoretic patterns obtained with haptoglobins using the starch gel technique Excess hemoglobin has been added to the plasma to complex the haptoglobins Benzidine has been used to show the presence of hemoglobin both bound and unbound The electrophoresis was carried out in 0 03 M borate buffer pH 8 5 6 volts per cm for 5 hr

For example it does not explain the absence of products of the type 2 2 homozygote in the heterozygous individual It does however suggest an approach which may relate the haptoglobin system to the one gene–one enzyme theory

In spite of the exceptions which have been taken to the one gene–one enzyme theory the essential correctness of the idea cannot be effectively challenged [82] One possibly might modernize it a bit by rephrasing it as one cistron–one polypeptide

## GENETIC INFLUENCES ON PROTEIN PROPERTIES

From the preceding sections it is obvious that virtually any property of a protein can be changed as a result of mutation The quantity of course is very frequently changed It will possibly be of some value at this point to consider some of the qualitative changes which have been demonstrated

In order for four alternative nucleotides to specify 20 amino acids a minimum of three nucleotide positions must be used to provide the 20 combinations [71] Three positions with four alternatives each give 64 possible combinations of which many may not specify an amino acid If a point mutation is pictured as a substitution of one nucleotide for another, then the new combination will sometimes be a "sense com bination and sometimes a 'nonsense combination [72] Since the latter will be unable to specify any amino acid at its appropriate point in the polypeptide chain then the polypeptide would be incomplete and might not be able to form at all This type of mutation may account for many of the mutants in which no product analogous to the unaltered protein can be detected

Implicit in much of what has been noted above is the assumption that each genetic locus is responsible for the synthesis of a specific protein This idea was effectively expressed by Beadle as the "one gene–one enzyme theory [73] This theory has been criticized for many reasons particularly in view of the developing concept that the dividing line between genes may be much less distinct than originally visualized in the ' beads-on a string picture Recent developments in understanding of hemoglobin structure and genetics are also relevant to this question First is the demonstration that each half molecule of hemoglobin con sists of two different polypeptide chains [74 75] Second is the report that abnormal hemoglobins S and G are not alleles as evidenced by the fact that a person heterozygous for both S and G produced a child which had only normal hemoglobin [76] This could have happened only if this G/S individual had a normal alternative for *each* of these abnormal genes These two facts taken together have led to the suggestion that the amino acid alterations in hemoglobin G and in hemoglobins S and C occur in different polypeptide chains and that each chain is controlled by a separate genetic locus [77] This would mean that at least two loci are involved in the production of a single complete molecule Consistent with the above argument is the observation that hemoglobins S and C involving an alteration at the same amino acid position are allelic [78]

Another system which superficially appears to contradict the one gene–one enzyme theory is the haptoglobins These are a group of glyco proteins found in human plasma and first detected by Polonovski and Jayle [79] Haptoglobins are unique because of their ability to combine irreversibly and stoichiometrically with hemoglobin Smithies [80] with the aid of his high resolution starch gel electrophoresis was able to dis tinguish three types of individuals each characterized by qualitative differences in his haptoglobins These three types are shown in Fig 2 9 A genetic analysis of family data by Smithies and Walker [81] indicated that this variation is controlled by a single locus with two alleles two of the types representing the two homozygotes and the third the heter

ozygote It is important to note that in this case a single locus controls the specificity of a whole series of proteins and furthermore that the heterozygous individuals produce a series of proteins (hybrid substances) differing from either homozygote The explanation of this unusual system has yet to come The interaction of the alleles in the heterozygote—difficult to visualize at the gene level—suggests that a series of polymers may be involved Thus each allele may control the production of a single monomer with subsequent reactions independent of this locus producing varying degrees of polymerization Consistent with this hypothesis is the recent observation of Smithies [81a] that treatment of haptoglobins with sulfhydryl reagents and urea produces protein subunits which correspond more closely to the genes present and may represent the monomeric forms This hypothesis does not explain all properties of the system

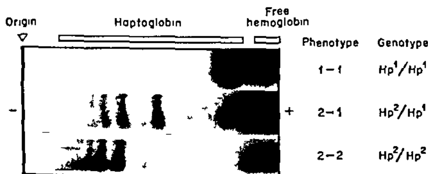



Fig 2 9 Plasma electrophoretic patterns obtained with haptoglobins using the starch gel technique Excess hemoglobin has been added to the plasma to complex the haptoglobins Benzidine has been used to show the presence of hemoglobin both bound and unbound The electrophoresis was carried out in 0 03 M borate buffer pH 8 5 6 volts per cm for 5 hr

For example it does not explain the absence of products of the type 2 2 homozygote in the heterozygous individual It does however suggest an approach which may relate the haptoglobin system to the one gene–one enzyme theory

In spite of the exceptions which have been taken to the one gene–one enzyme theory the essential correctness of the idea cannot be effectively challenged [82] One possibly might modernize it a bit by rephrasing it as one cistron–one polypeptide

## GENETIC INFLUENCES ON PROTEIN PROPERTIES

From the preceding sections it is obvious that virtually any property of a protein can be changed as a result of mutation The quantity of course is very frequently changed It will possibly be of some value at this point to consider some of the qualitative changes which have been demonstrated

*Solubility*

This property of the protein rarely changes enough with mutation to give rise to observable differences. The one striking exception occurs in man in the case of sickle cell hemoglobin. Although the oxygenated form of hemoglobin S is quite soluble, the nonoxygenated form is sparingly soluble, being only one fiftieth that of hemoglobin A [83]. In well oxygenated blood the hemoglobin S remains in solution, but if oxygen tension is low, the hemoglobin S will form "tactoids" inside the erythrocyte.

*Temperature Sensitivity*

The rate at which a protein undergoes "spontaneous" denaturation is a function of temperature. A number of cases are known in which temperatures compatible with life can cause relatively rapid denaturation of a particularly sensitive protein. One of the more carefully studied

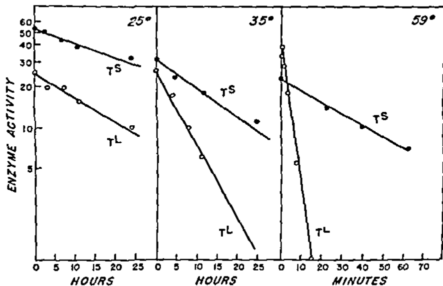



Fig. 2.10. Inactivation of thermolabile ($T^L$) and thermostable ($T^S$) tyrosinase (*Neurospora crassa*) at different temperatures. (*By permission of N. H. Horowitz and M. Fling* [84].)

occurs in *N. crassa* tyrosinase in a known mutant of this species is very sensitive to temperature. In their experiments with this mutant Horowitz and Fling obtained the inactivation graphs shown in Fig. 2.10 [84]. Other properties of the enzyme such as pH optimum, substrate specificity and sensitivity to cysteine were not altered.

Another example of a temperature sensitive mutant is the Himalaya rabbit, a domestic strain characterized by dark ears, tail, nose, and paws but white body fur [85]. If a portion of the skin is kept below 34 C the

body fur in that area will also be dark On the other hand if the animals are raised at temperatures greater than 34 C the entire animal will be white Again the explanation is the presence of a temperature sensitive pigment forming enzyme presumably tyrosinase This sensitivity is controlled by a single gene for which other alleles are known and which is recessive to the fully pigmented "wild type animal possessing stable tyrosinase

A possible example of temperature sensitivity has been reported in man As discu ed in Chap 32 approximately 10 per cent of male Negroes will undergo a hemolytic episode when exposed to primaquine and related compounds This trait is the result of a single gene located on the X chromosome [86 87] and has been traced to a deficiency of glucose 6-phosphate dehydrogenase in the erythrocytes [88] Studies of this enzyme in affected individuals have suggested that under certain con ditions it is more readily denatured by heat than is the enzyme from normal individuals [89] Such a mechanism would explain the self limiting nature of the hemolysis For example it has been established that only the older erythrocytes are sensitive to primaquine [90] Presumably the younger cells have a full complement of active enzyme which slowly becomes denatured leaving the older cells without defen e against the primaquine challenge

*Electrophoretic Mobility*

It is obvious that the introduction into proteins of different amino acids will frequently result in alteration of the total charge of the protein molecule and hence in mobility in an electric field The previous discussion of hemoglobin structure provides a good example of this Electrophoretic techniques have revealed two cases of genetic heterogeneity in man which were not previously suspected The first of these was haptoglobin the details of which have already been pre ented A more recent example is that of transferrin eight different types of tran ferrin have been demonstrated by means of the starch gel technique [91–94] Each of these types is the product of a single allele Homozygous individuals produce only a ingle type of tran ferrin whereas heterozygous individuals produce a mixture of two

*Immunologic Properties*

It is not po ible to di cu s in detail the many examples of inherited antigenic differences recognized in man Mo t of these occur on the sur faces of cells and have been largely refractory to chemical study but some progress has been made with the ABO antigens which do occur in a soluble form These largely carbohydrate ub tances have not yielded readily to studies of gene action (cf Kabat [95] for a review of this subject)

A recently described example of an inherited variation in a protein is the gamma factor of Grubb and Laurell [96, 97] which is recognized entirely by its interference with the ability of certain rheumatoid arthritic serums to promote the agglutination of Rh positive erythrocytes coated with incomplete Rh antibodies. This factor is found in the γ globulin fraction of serums and is either present or absent depending on the genotype of the individual. An immunologic difference has also been reported between hemoglobins A and S, although the magnitude was small [98].

*Sedimentation Rate*

An exchange of one amino acid for another in a large protein will not affect the molecular weight enough to alter the sedimentation rate in an ultracentrifuge but it may affect the configuration enough to influence the sedimentation and it may affect the formation of aggregates or polymers. Studies on the sedimentation of haptoglobins have revealed a small difference among the three types [99]. One would presume that the differences reflect different degrees of polymerization although whether this in turn is due to the protein or to the carbohydrate moiety is unknown. If it is due to the latter then it is probably not correct to consider this as an alteration in the primary gene product.

*Other Properties*

It is reasonable to assume that most measurable properties of proteins can be altered by mutation even though examples may not be known at present. Among the properties which should be considered are substrate specificity, Michaelis constant, pH optimum, etc. A distinct difference in any of these properties may be considered good evidence for a mutation.

## GENOTYPIC AND PHENOTYPIC EXPRESSION OF INHERITED TRAITS

### REGULATION OF PROTEIN SYNTHESIS

Whatever an individual's genetic make up may be, it is expressed only after interaction with the environment. It is often necessary to untangle complex environmental factors before one can bring order to the genetic elements. In addition, the gene products interact with each other, sometimes forming interlocking self regulating systems whose analysis may be extremely baffling. This section contains a discussion of a few of the systems which have been studied; it is hoped that they will serve as useful models for other systems.

If an organism carries the genetic information necessary to synthesize a specific protein, what then determines how much of that protein is

produced? The existence of regulating mechanisms is everywhere apparent but knowledge of them remains largely at the descriptive level One of the more interesting control phenomena is that of enzyme induction sometimes still referred to as adaptive enzyme formation [100] The ability of yeast to utilize galactose as a source of energy depends on the presence of an enzyme galactozymase Yeast cells grown on a glucose medium do not normally produce galactozymase but if galactose is supplied the galactozymase soon appears It is important to emphasize that this increase is not due to the selection of genetically different cells which already are producing the enzyme, rather it represents the new synthesis of galactozymase in individual cells which previously did not possess the enzyme In this sense the culture has become ' adapted to the medium although no selection has occurred

This phenomenon is more properly described as induction than 'adaptation This is illustrated by the experiments of Monod s group [101] In order for *E coli* to utilize lactose it must produce the enzyme $\beta$ galactosidase This enzyme is not ordinarily present in the absence of lactose but is produced rapidly if lactose is needed In addition to lactose several other galactosides can induce the formation of the enzyme one of which methyl $\beta$ D thiogalactoside is an even better inducer than lactose although it cannot serve as a substrate for the enzyme On the other hand phenyl $\beta$ D galactoside which does serve as a substrate cannot induce the synthesis of the enzyme With either of these substances as the sole carbon source *E coli* cannot grow with a mixture of the two—one to serve as inducer and the other as substrate—there is good growth This experiment is important in that it emphasizes that the inducer acts as a trigger mechanism only and does not alter the specificity of the enzyme produced

Studies by Novick and Weiner [102] again using induced $\beta$ galactosidase in *E coli* have shown that the process of induction is an all-or none phenomenon In a population of cells showing 50 per cent of maximum enzyme production half of the cells are fully induced and half are not induced This is explained on the basis of a second inducible enzyme located in the cell wall whose function is to concentrate the inducer inside the cell This enzyme permease is known to exist on the basis of other studies Normally it is present only in minute quantities if at all If inducer is added a cell which has some permease will begin to concentrate the inducer which then induces more permease Such a cell will maintain a much higher level of inducer within the cell and will remain fully induced for $\beta$ galactosidase in the presence of concentrations of inducer which were initially inadequate for induction

The extent to which induction is a factor in mammalian metabolism has not been determined A number of cases of enzyme adaptation have been reported tryptophan peroxidase is perhaps the most exten

A recently described example of an inherited variation in a protein is the gamma factor of Grubb and Laurell [96, 97] which is recognized entirely by its interference with the ability of certain rheumatoid arthritic serums to promote the agglutination of Rh positive erythrocytes coated with incomplete Rh antibodies This factor is found in the γ globulin fraction of serums and is either present or absent depending on the genotype of the individual An immunologic difference has also been reported between hemoglobins A and S, although the magnitude was small [98]

*Sedimentation Rate*

An exchange of one amino acid for another in a large protein will not affect the molecular weight enough to alter the sedimentation rate in an ultracentrifuge but it may affect the configuration enough to influence the sedimentation and it may affect the formation of aggregates or polymers Studies on the sedimentation of haptoglobins have revealed a small difference among the three types [99] One would presume that the differences reflect different degrees of polymerization although whether this in turn is due to the protein or to the carbohydrate moiety is unknown If it is due to the latter then it is probably not correct to consider this as an alteration in the primary gene product

*Other Properties*

It is reasonable to assume that most measurable properties of proteins can be altered by mutation even though examples may not be known at present Among the properties which should be considered are substrate specificity Michaelis constant pH optimum etc A distinct difference in any of these properties may be considered good evidence for a mutation

## GENOTYPE AND PHENOTYPE EXPRESSION OF INHERITED TRAITS

### REGULATION OF PROTEIN SYNTHESIS

Whatever an individual s genetic make up may be it is expressed only after interaction with the environment It is often necessary to untangle complex environmental factors before one can bring order to the genetic elements In addition the gene products interact with each other sometimes forming interlocking self regulating systems whose analysis may be extremely baffling This section contains a discussion of a few of the systems which have been studied, it is hoped that they will serve as useful models for other systems

If an organism carries the genetic information necessary to synthesize a specific protein what then determines how much of that protein is

## ENZYME BLOCK

Proteins are generally considered to have two functions—structural and enzymatic The number of enzymes known vastly exceeds the number of proteins with a recognized structural function and the production of inherited defect is generally considered to result from the interference with the enzymatic catalysis of some specific reaction In some cases the protein may be present but have no enzyme activity, and in others there may be no detectable protein product at all Examples of primary enzyme deficiencies in man include phenylketonuria (Chap 10) in which the phenylalanine hydroxylase fraction I is missing [105] and galactosemia (Chap 7) in which galactose 1 phosphate uridyl transferase is missing [106, 107]

A decrease in an enzymatically catalyzed reaction is not evidence that the specific enzyme is altered many examples exist in which other causes can be shown The production of an inhibitor by some other system may seriously interfere with a reaction In phenylketonuria the interference with normal pigmentation can be best explained as an inhibition of the pigment forming reactions by the greatly increased concentrations of phenylalanine or some of its unusual metabolites This is readily demonstrated by the deletion of phenylalanine from the diet of such individuals the subsequent increase in pigmentation indicates that the mechanisms for forming pigment are intact [108] Another example is found in galactosemia in which the accumulation of galactose 1 phosphate results in a marked aminoaciduria [109] (Chap 7) Transfer to a lactose-free diet causes the amino acid excretion to become normal In each of these examples the blocked reactions can also be deficient because of primary inherited defects in the respective enzymes

## PRODUCTION OF PHENOTYPES

Mendel in his studies of inherited variations in peas found certain characteristics to be *dominant* over their alternatives and others to be *recessive* to their alternatives Initially this was considered to be a property of the genetic determiner (gene) It is now apparent that the observer's interest is equally important at least as far as attaching a label to the gene This is illustrated very well by hemoglobin S [cf Chap 34] From the clinical point of view and ignoring the interaction with other rare hemoglobins only individuals who are homozygous for hemoglobin S are affected with sickle-cell disease This then conforms to the definition of a recessive trait On the other hand a person who looked only at erythrocyte preparations for the presence or absence of sickling under reducing conditions would consider the inheritance of this trait as dominant since heterozygous individuals also show the phenomenon A chemist looking at the composition of the hemoglobin would consider the alleles as lacking

sively studied [103]. The difficulty in demonstrating induced enzyme formation in higher animals stems from the fact that only in rare cases can one study a tissue not previously exposed to substrate. The differing enzyme capacities of differentiated tissues may well be due in part to differences in induction at early stages of embryonic development.

Certain tissues with secretory functions may produce more or less of a protein, depending on how much has been discharged from the cell. Such a circumstance can be explained by the effects of mass action, but proof is lacking on this point. In other situations the amount of substrate or product is an important regulating factor. An example is found in sickle cell disease, where the synthesis of fetal hemoglobin is increased because of the inability of nonfetal hemoglobin to keep tissues adequately oxygenated.

A potentially important aspect of the regulation of protein formation is the problem of production of nonfunctional protein at a mutant locus. It is known from the many studies on hemoglobin and the recent ones of transferrin [91] that the gene products from homologous loci on different chromosomes are usually produced independently. Haptoglobin is an apparent exception, but a mechanism has been suggested above which is compatible with the idea of two primary gene products. If the protein itself plays a role in determining how much product shall be made, then a protein which is "nonsense" as far as particular enzyme function is concerned may be "sense" as far as regulating the amount of product is concerned. The similarity of CRM to tryptophan synthetase bears out this thought. Should this prove to be the case, then heterozygous individuals who produce a nonsense protein may show a greater departure from normality than those who produce no protein at the mutant locus. F. Vogel (personal communication) has suggested this as a possible explanation for certain sex-linked genes in man. For example, classical hemophilia is a severe disease in *hemizygous* males (containing only one X chromosome) but shows no effect in heterozygous females. Some pedigrees are known, however, in which heterozygous females do show minor abnormalities although the hemizygous males are less severely affected than usual [104]. In such pedigrees it is not obvious why a male with one normal allele is entirely normal, while a female with one normal and one abnormal shows an effect of the abnormal. Interaction of the chromosomes themselves seems a most unlikely explanation. If one assumes that the synthesis of protein is determined by the total amount of product formed, then the normal allele may well be operating at considerably less than maximum capacity, since the abnormal protein would also count as product. The crucial test here would be the detection in heterozygous individuals of a nonfunctional cross-reacting product. Such a mechanism may also be operating in some dominantly inherited diseases.

or alteration The analysis by Gruneberg [113] of a gene resembling achondroplasia in the rat can serve as an excellent example of the common origin of pleiotropic effects A similar analysis of sickle cell disease in man relates the symptoms of impaired mental function poor physical development rheumatism fibrosis of spleen etc to the single change in the hemoglobin S molecule [114] It seems best to consider that all effects of a single gene are due to an alteration in a single primary protein at least until clear evidence to the contrary is available

Since mutant genes exert their effects by altering the rate of a specific chemical reaction it is not surprising that nongenetic events can also lead to the production of a phenotype which is indistinguishable from an inherited defect Such *phenocopies* occur primarily as developmental anomalies and probably represent differences in organization and timing of developmental processes rather than in the biochemical potential of the organism In higher animals the relatively constant temperature and chemical environment which the mother provides for the developing embryo act as strong buffers against nongenetic aberrations and their occurrence in man is probably more infrequent than in lower organisms Where they are thought to occur the biochemical mechanism for the genetic form is obscure and a comparative study at the biochemical level has not been possible Presumably the enzyme levels would show little variation from normal Diseases recognized as enzyme deficiencies and expressed only after birth such as galactosemia and phenylketonuria possibly never occur as phenocopies

## DIFFERENTIATION

It is evident that the nuclei of germ cells contain all the chemical potential of all the cells which will subsequently differentiate into tissues of differing chemical function The question of how certain potentials are developed and others are repressed during differentiation has long been an intriguing one most of the answers are still to be ascertained Since it is difficult to imagine the nucleus as gaining functions which it did not previously possess it is generally assumed that differentiation represents a selective loss of functions or a failure to develop functions for which the necessary information was present in the original germ cell To what extent are these losses reversible? In plants at least they seem to be largely reversible as evidenced by the ability of differentiated tissues to give rise to complete plants This ability is not wide spread in animals however and the early embryologists had evidence that was consistent with the idea that after the first divisions of a fertilized egg only the germinal tissues retained a complete nuclear complement

The recent studies of King and Briggs [115] have done much to elucidate this question They have transplanted nuclei from differentiated tissues of frog embryos at different stages of development into enucleated eggs

dominance since each allele can synthesize its specific hemoglobin regardless of the associated allele

Phenylketonuria also provides an example in which the abnormal gene which produces disease only in the homozygous combination is detectable in heterozygous combination Carriers of this disease have a slightly higher blood level of phenylalanine than do normal persons and they have an abnormal phenylalanine tolerance test result [110, 111] The gene therefore is recessive as far as the disease is concerned but not as far as a change in phenylalanine metabolism is concerned

A further point may be raised about many of the rare dominant diseases They are generally regarded as situations in which the presence of only one abnormal gene is sufficient for expression of *the disease* Actually because of the rarity of these genes it is seldom known what their expression would be in the homozygous combination

The terms *dominant* and *recessive* will continue to be useful descriptions of the expressions of different alleles In using them one should remember that they are related to a particular type of observation and that particular findings result from the interaction of the gene with other systems

Variations of other systems both genetic and nongenetic may influence considerably the intensity of expression of a particular gene in some cases enhancing its effects and in others deleting its effects entirely In working with nonpurebred strains such as man the variation in genetic expression is apt to be particularly evident In many cases a mutant trait will not be expressed at all in an individual who is shown to have the mutant genotype by pedigree analysis Genes which are always detectable if present in the correct combination (heterozygous for dominants, homozygous for recessives) are said to be completely *penetrant* Genes which do not always produce the detectable end result are said to have lowered penetrance Although penetrance can sometimes be used to distinguish between two mutant genes having the same end result the level of penetrance will vary with the level of observation just as in dominant versus recessive traits Thus a child with genes for galactosemia will not develop the disease on a lactose free diet [112] If such diets were prevalent then the penetrance of galactosemia would be much lower than it in fact is On the other hand galactosemic individuals reared on a lactose free diet still have an abnormal galactose tolerance test result and if such a test were the criterion for the trait then the penetrance would be complete

Many genes produce a variety of multiple effects involving functions not otherwise known to be related The term *pleiotropism* has been used to describe this phenomenon It is difficult to reconcile a true multiple gene action with the one gene–one enzyme theory discussed earlier and in those cases of pleiotropism which have been adequately analyzed, all the observed changes can be traced ultimately to a single primary defect

or alteration The analysis by Gruneberg [113] of a gene resembling achondroplasia in the rat can serve as an excellent example of the common origin of pleiotropic effects A similar analysis of sickle cell disease in man relates the symptoms of impaired mental function poor physical development rheumatism fibrosis of spleen etc to the single change in the hemoglobin S molecule [114] It seems best to consider that all effects of a single gene are due to an alteration in a single primary protein at least until clear evidence to the contrary is available

Since mutant genes exert their effects by altering the rate of a specific chemical reaction it is not surprising that nongenetic events can also lead to the production of a phenotype which is indistinguishable from an inherited defect Such *phenocopies* occur primarily as developmental anomalies and probably represent differences in organization and timing of developmental processes rather than in the biochemical potential of the organism In higher animals the relatively constant temperature and chemical environment which the mother provides for the developing embryo act as strong buffers against nongenetic aberrations and their occurrence in man is probably more infrequent than in lower organisms Where they are thought to occur the biochemical mechanism for the genetic form is obscure and a comparative study at the biochemical level has not been possible Presumably the enzyme levels would show little variation from normal Diseases recognized as enzyme deficiencies and expressed only after birth such as galactosemia and phenylketonuria possibly never occur as phenocopies

## DIFFERENTIATION

It is evident that the nuclei of germ cells contain all the chemical potential of all the cells which will subsequently differentiate into tissues of differing chemical function The question of how certain potentials are developed and others are repressed during differentiation has long been an intriguing one most of the answers are still to be ascertained Since it is difficult to imagine the nucleus as gaining functions which it did not previously possess it is generally assumed that differentiation represents a selective loss of functions or a failure to develop functions for which the necessary information was present in the original germ cell To what extent are these losses reversible? In plants at least they seem to be largely reversible as evidenced by the ability of differentiated tissues to give rise to complete plants This ability is not widespread in animals however and the early embryologists had evidence that was consistent with the idea that after the first divisions of a fertilized egg only the germinal tissues retained a complete nuclear complement

The recent studies of King and Briggs [115] have done much to elucidate this question They have transplanted nuclei from differentiated tissues of frog embryos at different stages of development into enucleated egg

and have then studied the ability of these eggs to develop into embryos Their experiments clearly demonstrate that nuclei from early embryos can give rise to normal embryos when transplanted The older the donor embryo the more abnormalities were observed Even if the transplanted nuclei were obtained from the late gastrula stage a few normal embryos were obtained Thus the genetic information had remained intact in at least some of the cells through this stage of development It was also noted however that nuclei transplanted from a particular donor tissue tended to promote development of that tissue in the recipient egg suggesting that some type of nuclear differentiation possibly had occurred

Another example of the role of genotype in differentiation is found in the work of Stern [*116*] using *Drosophila* It is possible to obtain genetic *mosaics* in *Drosophila* in which parts of the tissues of an individual differ by one or more genes from the remainder of the fly Events which occur at some specific place such as the formation of a bristle may be inhibited by a genotype for absence of bristles at that place If tissue containing normal genotype is not too distant then the bristle may form at the site of this normal tissue a place where it would never form in a normal fly Thus the genetic potential for bristle formation is retained by tissue which is not required to form bristles during normal development

Such studies neither prove nor disprove the hypothesis that the genetic information remains intact in all the cells of a fully differentiated animal They do prove that factors other than selective genetic loss are responsible for the early stages of differentiation and suggest that such factors by their ability to inhibit the function of certain genes are responsible for all differentiation

## MODES OF INHERITANCE—ANALYSIS OF PEDIGREES

The operational nature of such terms as dominance recessiveness and penetrance has been pointed out Nevertheless these terms have a very real meaning in the prediction of the recurrence of inherited traits Such predictions are commonly used for two purposes—in genetic counseling and in the understanding of genetic mechanisms The discussions in this section will be concerned with the latter

The analysis of pedigrees can be a potent tool in the recognition of identity or nonidentity of mutant genes One example is in the differentiation of two genes on the basis of their linkage groups In man this normally consists in recognition that one of the genes is transmitted on the X chromosome while the other is transmitted on an autosome This distinction has been useful in recognizing different forms of muscular dystrophy and some of the blood clotting disorders A single example exists in which autosomal linkage has been used to distinguish two abnormal genes otherwise similar in their manifestations This is the

case of elliptocytosis linked to the Rh blood type in some but not all pedigrees [14] It is to be expected that as more markers become available for human chromosomes this approach will become much more powerful than it is at present

A second case in which it is possible to recognize the existence of more than one form of abnormal gene is that in which the forms show different epistatic relationships (i e dominance or recessiveness) This is illustrated by the work of Harris Mittwoch Robson and Warren on cystinuria [117] In one form of the disease the carriers show no abnormality in their urinary excretion of cystine and lysine [Chap 42] In the other form the carriers can be detected by their increased excretion of cystine and lysine Thus one type is recessive and the other incompletely recessive The homozygous individuals are similar in the two forms of the disease This evidence that two types of abnormal gene exist does not tell us whether these two types are allelic or not Thus these two forms of cystinuria may represent mutations of two functional genetic loci located on different chromosomes or they may be alleles one of which is slightly more deleterious in its action

Families in which more than one recognizable gene is segregating can sometimes yield much information on genetic mechanisms Thus one parent cannot pass two alleles to the same offspring If two genes are passed from parent to offspring then this is sufficient evidence for their being nonallelic Again nonallelism is indicated if neither gene is transmitted to an offspring This is illustrated by the family already described in which hemoglobins S and G were shown to be nonallelic In such critical cases it is obviou ly important to e tablish correctly the family relation hips

In the remainder of this chapter are reviewed some of the simple forms of inheritance

## CHROMOSOMAL INHERITANCE

Virtually all recognized genetic variation is Mendelian in that it involves genetic determiners located on the chromosomes The e determiners are transmitted to the off pring in a regular manner as outlined at the beginning of this chapter resulting in only a few well-defined modes of inheritance These different modes are caused by differences in dominance versus rece ivene s and in auto omal versus sex linkage The common patterns of inheritance are outlined in the following sections

### *Autosomal Dominant Inheritance*

A gene which is fully expressed when in the heterozygous combination is said to be dominant and if it occurs on other than the sex chromosomes it is said to be auto omal At lea t one parent of any affected individual will also carry the gene and if the gene has high penetrance that parent

will also be affected A gene may be expressed in only one sex but if it is an autosomal dominant, then it will be *transmitted* by parents of either sex to children of either sex If the gene is rare then usually only one parent will be affected Approximately half the children of an affected person will also be affected Occasionally a mutation may occur giving

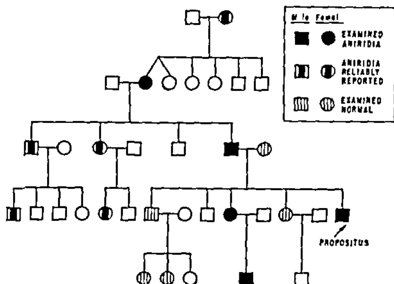



Fig 2 11 A pedigree of aniridia illustrating autosomal dominant pattern of inheritance

rise to an affected individual from unaffected parents A pedigree of a simple dominant trait is shown in Fig 2 11

*Autosomal Recessive Inheritance*

If a trait is expressed only in the homozygous combination then it is recessive This common mode of inheritance for diseases of enzymatic defect is characterized as follows (1) For a rare gene the affected individuals in a family will nearly always be in a single sibship (2) For a rare gene the parents of an affected individual will be relatives more often than in the general population (3) Approximately one fourth of the siblings of an affected individual will also be affected (4) Homozygous males and females should occur in equal numbers although the expression may vary greatly with sex A pedigree of a simple recessive trait is shown in Fig 2 12

*Sex linked Dominant Inheritance*

Conventionally a mutant gene is thought to have a decreased ability to carry out a function Since a normal male has only one X chromosome a female who has only one mutant gene on an X chromosome has the

same number of normal genes as a male and should be able to function as well Using this line of argument sex linked dominants should not exist at all They are in fact exceedingly rare A discussion of their possible biochemical mechanism has already been given (see Regulation of Protein Synthesis above) For such a model of inheritance, (1) affected males will always have an affected mother while affected females will have an affected father one third of the time and an affected mother two thirds of the time (2) The daughters of an affected male will all be

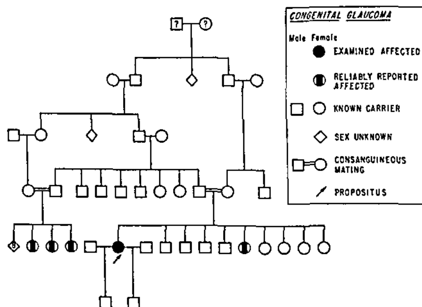



Fig 2 12 A pedigree of congenital glaucoma illustrating autosomal recessive pattern of inheritance The individual marked as carriers are known to be such because of their relationship to others in the pedigree Some of the individuals not marked are undoubtedly carriers but this cannot be demonstrated

affected while none of the sons will be affected (3) An affected female will *transmit the trait to half her sons and half her daughters* (4) Since females have twice as many X chromosomes as do males there will be twice as many affected females in the population as there are males An example of sex linked dominant inheritance is found in Fig 6-7

## *Sex linked Recessive Inheritance*

This mode of inheritance has long been recognized in man because of several unique properties In males a single mutant gene will be expressed since there is no normal allele to function In females on the

other hand the mutant gene must be present on both X chromosomes in order for the trait to be expressed Thus (1) affected males will *never* transmit the trait to their sons but all their daughters will be heterozygous "carriers" (2) Heterozygous females will transmit the mutant gene to half their sons who will be affected and to half their daughters who will be carriers (3) If an affected male marries a carrier female then half their offspring will be affected regardless of sex (4) The sons of affected females will always be affected (5) For sex linked recessive traits more males will be affected than females the rarer the gene, the higher the ratio of males to females

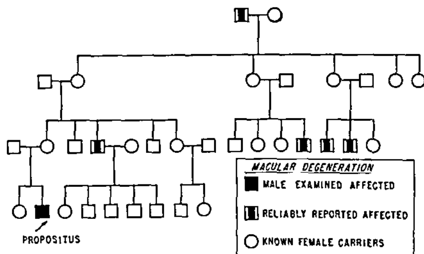



Fig 2 13 A pedigree of macular degeneration illustrating sex linked recessive pattern of inheritance The females marked as carriers are known to be such because of their relationship to affected males Some of the other females may also be carriers

An example of a pedigree showing sex linked recessive inheritance is given in Fig 2 13 It is necessary to distinguish between sex linked inheritance and sex limited inheritance In the latter case the trait is expressed only in one sex but the pattern of inheritance is that of an autosomal gene Such a gene can be transmitted from father to son a mode never found in sex linked inheritance

*Inheritance on the Y Chromosome*

The Y chromosome has been envisioned as consisting of two segments—one homologous with a portion of the X chromosome and the other non homologous with the X chromosome The sex linked genes already discussed would occur on the nonhomologous portion of the X chromosome Genes occurring on the homologous portions of the sex chromosomes are referred to as 'partially sex linked' and genes occurring on the non homologous portion of the Y chromosome are referred to as holandric

or Y linked Haldane [118] first suggested the existence of partial sex linkage in man but subsequent attempts to verify it have been unsuccessful and it is now considered doubtful that there exists a homologous segment at all

There are a number of pedigrees in the literature for which holandric inheritance is the most probable explanation Stern [12] in a masterful piece of detective work has shown the need for revision of some of the more striking of these pedigrees with the result that they become more compatible with other modes of inheritance Although he is duly cautious about excluding the possibility of complete Y linkage he very effectively points out the inadequacy of the evidence for its existence In complete Y linkage affected males will transmit the trait to all their sons and to none of their daughters It is necessary to distinguish this mode of inheritance from an autosomal dominant trait which is expressed only in males In the latter case half the daughters of an affected male though unaffected themselves would be able to transmit the trait to their sons

## CYTOPLASMIC INHERITANCE

The long-debated question of the presence of genetic determiners in the cytoplasm has in recent years been resolved in their favor [119 120] The demonstration of such a phenomenon rests on the premise that spermatozoa carry with them virtually no cytoplasm and hence cannot transmit the trait in question while eggs from a particular individual will all carry the same information in their cytoplasm Thus all the progeny would resemble the female parent and none the male parent in a mating between two individuals differing in some cytoplasmic factor This expectation is quite unlike any for traits whose variation is controlled by the nucleus

One of the more striking demonstrations of cytoplasmic inheritance occurs in paramecia [121] Certain of these organisms elaborate a substance into the medium which is toxic for other individuals The former killer paramecia were shown to transmit the trait through their cytoplasm and the trait has been shown to be due to the presence of particles in the cytoplasm of killer cell These particles are about 0 4 micron in diameter and they stain with the Feulgen stain which indicates their deoxyribonucleic acid nature They are capable of replicating and being transmitted by the cytoplasm Their maintenance however requires the presence in the nuclear genome of a particular dominant gene K and they cannot be transmitted to cells which lack K Cells which lack the kappa particles in their cytoplasm are sensitive to its toxic effects regardless of their genotype

Other examples of cytoplasmic inheritance have been found in *Neurospora* of which the *poky* strain was the first observed [122] This strain is characterized by very slow rate of growth although in a contained cul

other hand the mutant gene must be present on both X chromosomes in order for the trait to be expressed Thus (1) affected males will *never* transmit the trait to their sons but all their daughters will be heterozygous "carriers" (2) Heterozygous females will transmit the mutant gene to half their sons who will be affected and to half their daughters who will be carriers (3) If an affected male marries a carrier female then half their offspring will be affected regardless of sex (4) The sons of affected females will always be affected (5) For sex linked recessive traits more males will be affected than females, the rarer the gene the higher the ratio of males to females

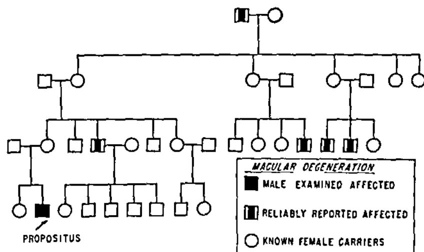



Fig 2 13 A pedigree of macular degeneration illustrating sex linked recessive pattern of inheritance The females marked as carriers are known to be such because of their relationship to affected males Some of the other females may also be carriers

An example of a pedigree showing sex linked recessive inheritance is given in Fig 2 13 It is necessary to distinguish between sex linked inheritance and sex limited inheritance In the latter case the trait is expressed only in one sex but the pattern of inheritance is that of an autosomal gene Such a gene can be transmitted from father to son a mode never found in sex linked inheritance

*Inheritance on the Y Chromosome*

The Y chromosome has been envisioned as consisting of two segments—one homologous with a portion of the X chromosome and the other nonhomologous with the X chromosome The sex linked genes already discussed would occur on the nonhomologous portion of the X chromosome Genes occurring on the homologous portions of the sex chromosomes are referred to as "partially sex linked" and genes occurring on the nonhomologous portion of the Y chromosome are referred to as holandric

or Y linked Haldane [118] first suggested the existence of partial sex linkage in man but subsequent attempts to verify it have been unsuccessful and it is now considered doubtful that there exists a homologous segment at all

There are a number of pedigrees in the literature for which holandric inheritance is the most probable explanation Stern [12] in a masterful piece of detective work, has shown the need for revision of some of the more striking of these pedigrees with the result that they become more compatible with other modes of inheritance Although he is duly cautious about excluding the possibility of complete Y linkage he very effectively points out the inadequacy of the evidence for its existence In complete Y linkage affected males will transmit the trait to all their sons and to none of their daughters It is necessary to distinguish this mode of inheritance from an autosomal dominant trait which is expressed only in males In the latter case half the daughters of an affected male though unaffected themselves would be able to transmit the trait to their sons

## CYTOPLASMIC INHERITANCE

The long-debated question of the presence of genetic determiners in the cytoplasm has in recent years been resolved in their favor [119, 120] The demonstration of such a phenomenon rests on the premise that spermatozoa carry with them virtually no cytoplasm and hence cannot transmit the trait in question while eggs from a particular individual will all carry the same information in their cytoplasm Thus all the progeny would resemble the female parent and none the male parent in a mating between two individuals differing in some cytoplasmic factor This expectation is quite unlike any for traits whose variation is controlled by the nucleus

One of the more striking demonstrations of cytoplasmic inheritance occurs in paramecia [121] Certain of these organisms elaborate a substance into the medium which is toxic for other individuals The former killer paramecia were shown to transmit the trait through their cytoplasm and the trait has been shown to be due to the presence of particles in the cytoplasm of killer cells These particles are about 0 4 micron in diameter and they stain with the Feulgen stain which indicates their deoxyribonucleic acid nature They are capable of replicating and being transmitted by the cytoplasm Their maintenance however, requires the presence in the nuclear genome of a particular dominant gene K and they cannot be transmitted to cells which lack K Cells which lack the kappa particles in their cytoplasm are sensitive to its toxic effects regardless of their genotype

Other examples of cytoplasmic inheritance have been found in *Neurospora* of which the *poky* strain was the first observed [122] This strain is characterized by very slow rate of growth although in a contained cul

ture the final growth approaches that of the wild type A mating of poky female (protoperithecium) by wild type male (conidia) produces only poky offspring while the reciprocal mating produces only wild type offspring The trait was found to be caused by the presence of abnormal mitochondria which among other differences, have an unusual cytochrome composition

Mitochondria, as well as other particles replicate in the cytoplasm and are then distributed randomly when a cell divides The demonstration of a transmitted variation influenced by the nucleus raises questions as to the nature of the role which the nucleus plays Does it have primarily a "permissive" function, with most of the genetic specificity residing in the multitude of cytoplasmic particles or does it actually supply most of the detailed information necessary for the many synthetic processes of the cell with the variation in cytoplasmic factors occurring only as a rare event? The evidence favors the latter view First cytoplasmic inheritance has not been often detected and has never been demonstrated in higher animals Second the detailed amino acid composition of proteins such as hemoglobin is controlled by factors which segregate in a Mendelian fashion It seems best to regard cytoplasmic inheritance as an infrequent occurrence particularly in man but one which must be considered in the genetic analysis of variation

## BIBLIOGRAPHY


1 Painter T S Studies in mammalian spermatogenesis II The spermatogenesis of man J Exper Zool 37 291 1923
2 Tjio J H and Levan A The chromosome number of man Hereditas 42 1 1956
3 Tjio J H and Puck T T Genetics of somatic mammalian cells II Chromosomal constitution of cells in tissue culture J Exper Med 108 259 1958
4 Chu E H Y and Giles N H Human chromosome complements in normal somatic cells in culture Am J Human Genet 11 63 1959
5 Kodani M Three chromosome numbers in whites and Japanese Science 127 1339 1958
6 Kodani M The supernumerary chromosome of man Am J Human Genet 10 125 1958
7 Jacobs P A and Strong J A A case of human intersexuality having a possible XXY sex-determining mechanism Nature London 183 302 1959
8 Ford C E Polani P E Briggs J H and Bishop P M F A presumptive human XXY/XX mosaic Nature London 183 1030 1959
9 Stern C Colourblindness in Klinefelter s syndrome Nature London 183 145 1959
10 Lejeune J Gautier M and Turpin R Les chromosomes humains en culture de tissus C R Acad Sci Paris 248 602 1959
11 Ford C E Jones K W Miller O J Mittwoch U Penrose L S Ridler M and Shapiro A The chromosomes in a patient showing both mongolism and the Klinefelter syndrome Lancet 1 709 1959
12 Stern C The problem of complete Y linkage in man Am J Human Genet 9 147 1957
13 Lawler S D and Sandler M Data on linkage in man elliptocytosis and blood groups IV Families 5 6 and 7 Ann Eugenics 18 328 1954

14 Morton N E The detection and estimation of linkage between the genes for elliptocytosis and the Rh blood type Am J Human Genet 8 80 1956
15 Renwick J H and Lawler S D Genetical linkage between the ABO and nail patella loci Ann Human Genet 19 31? 1955
16 Mohr J *A Study of Linkage in Man* Munksgaard Copenhagen 1954
17 Sanger R and Race R R The Lutheran-secretor linkage in man support for Mohr s findings Heredity 12 513 1958
18 Goodall H B Hendry D W W Lawler S D and Stephen S A Data on linkage in man elliptocytosis and blood groups III Family 4 Ann Eugenics 18 325 1954
19 Avery O T MacLeod C M and McCarty M Studies on the chemical nature of the substance inducing transformation of pneumococcal types J Exper Med 79 137 1944
20 Griffith F The significance of pneumococcal types J Hyg 27 113 19?8
?1 Hotchkiss R D The role of desoxyribonucleates in bacterial transformations in *Phosphorus Metabolism* edited by W D McElroy and B Glass vol II pp 4?6–436 Johns Hopkins Press Baltimore 195_
22 Zamenhof S Alexander H E and Leidy G Studies on the chemistry of the transforming activity I Resistance to physical and chemical agents J Exper Med 98 3?3 19?3
23 Hershey A D and Chase M Independent functions of viral protein and nucleic acid in growth of bacteriophage J Gen Physiol 36 39 1952
24 Fraenkel Conrat H Singer B A and Williams R C The nature of the progeny of virus reconstituted from protein and nucleic acid of different strains of tobacco mosaic virus in *The Chemical Basis of Heredity* edited by W D McElroy and B Glass pp 501–51 Johns Hopkins Press Baltimore 1957
25 Gierer A and Schramm G Infectivity of ribonucleic acid from tobacco mosaic virus Nature London 177 ?0? 1956
26 Wyatt G R and Cohen S S The bases of the nucleic acids of some bacterial and animal viruses The occurrence of 5 hydroxymethyl-cytosine Biochem J 55 774 1953
?7 Watson J D and Crick F H C A structure for deoxyribose nucleic acid Nature London 171 737 1953
28 Wilkins M H F Physical studies of the molecular structure of deoxyribose nucleic acid and nucleoprotein Cold Spring Harbor Symposia Quant Biol 21 75 1956
?9 Watson J D and Crick F H C Genetical implications of the structure of deoxyribonucleic acid Nature London 171 964 1953
29a Sinsheimer R L Single-stranded deoxyribonucleic acid from bacteriophage φX174 J Mol Biol 1 43 1959
30 Lewis E B Pseudoallelism and gene evolution Cold Spring Harbor Symposia Quant Biol 16 151 1951
31 Pontecorvo G *Trends in Genetic Analysis* Columbia University Press New York 1958
32 Benzer S Fine structure of a genetic region in bacteriophage Proc Nat Acad Sc (U S) 41 344 1955
33 Benzer S The elementary units of heredity in *The Chemical Basis of Heredity* edited by W D McElroy and B Glass pp ?0–?3 Johns Hopkins Press Baltimore 1957
34 Benzer S and Freese E Induction of specific mutations with 5-bromouracil Proc Nat Acad Sc (U S) 44 11? 1958
34a Davison P F The effect of hydrodynamic shear on the deoxyribonucleic acid from T and T bacteriophages Proc Nat Acad Sc (U S) 45 1560 1959
35 Levinthal C and Crane H R On the unwinding of DNA Proc Nat Acad Sc (U S) 42 43? 1956
3? Levinthal C The mechanism of DNA replication and genetic recombination in phage Proc Nat Acad Sc (U S) 42 394 1956
37 Taylor J H Woods P S and Hughes W L The organization and duplication

of chromo omes as revealed by autoradiographic studies using tritium labeled thymidine Proc Nat Acad Sc (U S) 43 1 ' 195"

37a Meselson M and Stahl F W The replication of DNA in *Escherichia coli* Proc Nat Acad Sc (U S) 44 671 1958

38 Caspersson T Studien über den Eiweissumsatz der Zelle Naturwissenschaften 29 33 1941

39 Brachet J La localisation des acides pentosenucléiques dans les tissus animaux et les oeufs d Amphibiens en voie de développement Arch Biol (Liége) 53 207 1942

40 Vendrely R The deoxyribonucleic acid content of the nucleus in *The Nucleic Acids* edited by E Chargaff and J N Davidson vol 2 pp 155–180 Academic Press Inc New York 1955

41 Brachet J *Biochemical Cytology* Academic Press Inc New York 1957

42 Lipmann F Metabolic generation and utilization of phosphate bond energy Advances Enzymol 1 99 1941

43 Hoagland M B An enzymic mechanism for amino acid activation in animal tissues Biochim et biophys acta 16 288 1955

44 Hoagland M B Keller E B and Zamecnik P C Enzymatic carboxyl activation of amino acids J Biol Chem 218 345 1956

45 Kingdon H S Webster L T Jr and Davie E W Enzymatic formation of adenyl tryptophan isolation and identification Proc Nat Acad Sc (U S) 44 757 1958

46 Zamecnik P C Stephenson M L and Hecht L I Intermediate reactions in amino acid incorporation Proc Nat Acad Sc (U S) 44 73 1958

47 Lipmann F Symposium on amino acid activation Chairman s introduction Some facts and problems Proc Nat Acad Sc (U S) 44 67 1958

48 Hoagland M B Zamecnik P C and Stephenson M L Intermediate reactions in protein biosynthesis Biochim et biophys acta 24 215 1957

49 Berg P and Ofengand E J An enzymatic mechanism for linking amino acids to RNA Proc Nat Acad Sc (U S) 44 78 1958

50 Schweet R S Bovard F C Allen E and Glassman E The incorporation of amino acids into ribonucleic acid Proc Nat Acad Sc (U S) 44 173 1958

51 Hecht L I Stephenson M L and Zamecnik P C Dependence of amino acid binding to soluble ribonucleic acid on cytidine triphosphate Biochim et biophys acta 29 460 1958

52 Zachau H G Acs G and Lipmann F Isolation of adenosine amino acid esters from a ribonuclease digest of soluble liver ribonucleic acid Proc Nat Acad Sc (U S) 44 885 1958

53 Hecht L I Zamecnik P C Stephenson M L and Scott J F Nucleoside triphosphates as precursors of ribonucleic acid end groups in a mammalian system J Biol Chem 233 954 1958

54 Borsook H Deasy C L Haagen Smit A J Keighley G and Lowy P H Metabolism of C labeled glycine L-histidine L-leucine and L-lysine J Biol Chem 187 839 1950

55 Zamecnik P C and Keller E B Relation between phosphate energy donors and incorporation of labeled amino acids into proteins J Biol Chem 209 337 1954

56 Littlefield J W Keller E B Gross J and Zamecnik P C Studies on cytoplasmic ribonucleoprotein particles from the liver of the rat J Biol Chem 217 111 1955

57 Keller E B and Zamecnik P C The effect of guanosine diphosphate and triphosphate on the incorporation of labeled amino acids into proteins J Biol Chem 221 45 1956

58 McLean J R Cohn G L Brandt I K and Simpson M V Incorporation of labeled amino acids into the protein of muscle and liver mitochondria J Biol Chem 233 657 1958

59 Spiegelman S Nucleic acids and the synthesis of protein in *The Chemical Basis of Heredity* edited by W D McElroy and B Glass pp 232–267 Johns Hopkins Press Baltimore 1957

60 Hoagland M B Stephenson M L Scott J F Hecht L I and Zamecnik P C A soluble ribonucleic acid intermediate in protein synthesis J Biol Chem 231 241 1958
61 Goldstein L and Plaut W Direct evidence of nuclear synthesis of cytoplasmic ribonucleic acid Proc Nat Acad Sc (U S) 41 874 1955
62 Plaut W and Rustad R C Uptake of adenine 8- C by whole and fractional amoebae Nature London 177 89 1956
63 Borsook H Deasy C L Haagen Smit A J Keighley G and Lowy P H Incorporation *in vitro* of labeled amino acids into proteins of rabbit reticulocytes J Biol Chem 196 669 1952
64 Allfrey V G Mirsky A E and Osawa S Protein synthesis in isolated cell nuclei J Gen Physiol 40 451 1957
65 Markert C L and Owen R. D Immunogenetic studies of tyrosinase specificity Genetics 39 818 1954
66 Suskind S R Yanofsky C and Bonner D M Allelic strains of *Neurospora* lacking tryptophan synthetase a preliminary immunochemical characterization Proc Nat Acad Sc (U S) 41 577 1955
66a Lederberg J Genes and antibodies Science 129 1649 1959
67 Schroeder W A and Matsuda G N terminal residues of human fetal hemoglobin J Am Chem Soc 80 1521 1958
68 Ingram V M A specific chemical difference between the globins of normal human and sickle-cell anaemia haemoglobin Nature London 178 792 1956
69 Hunt J A and Ingram V M Allemorphism and the chemical differences of the human haemoglobins A S and C Nature London 181 1062 1958
70 Benzer S Ingram V M and Lehmann H Three varieties of human haemoglobin D Nature London 182 852 1958
71 Gamow G Possible relation between deoxyribonucleic acid and protein structure Nature London 173 318 1954
72 Crick F H C Griffith J S and Orgel L E Codes without commas Proc Nat Acad Sc (U S) 43 416 1957
73 Beadle G W Biochemical genetics Chem Rev 37 15 1945
74 Rhinesmith H S Schroeder W A and Pauling L A quantitative study of the hydrolysis of human dinitrophenyl(DNP)globin the number and kind of polypeptide chains in normal adult human hemoglobin J Am Chem Soc 79 4682 1957
75 Rhinesmith H S Schroeder W A and Martin N The N terminal sequence of the β chains of normal adult human hemoglobin J Am Chem Soc 80 3358 1958
76 Schwartz H C Spaet T H Zuelzer W W Neel J V Robinson A R and Kaufman S F Combinations of hemoglobin G hemoglobin S and thalassemia occurring in one family Blood 12 238 195
77 Neel J V Aspects of the genetic control of the structure of the hemoglobin molecule *Proc X International Congress of Genetics* vol 1 p 108 1958
78 Ranney H M Observations on the inheritance of sickle-cell hemoglobin and hemoglobin C J Clin Invest 33 1634 1954
79 Polonovski M and Jayle M Existence dans le plasma sanguin d une substance activant l action peroxydasique de l hémoglobine Compt rend soc biol 129 457 1938
80 Smithies O Grouped variations in the occurrence of new protein components in normal human serum Nature London 175 307 1955
81 Smithies O and Walker N F Genetic control of some serum proteins in normal humans Nature London 176 1265 1955
81a Smithies O in *First Conference on Genetics* edited by H F Sutton Josiah Macy Jr Foundation New York 1960
82 Horowitz N H and Fling M The role of the genes in the synthesis of enzymes in *Enzymes Units of Biological Structure and Function* edited by O H Gaebler pp 139–145 Academic Press Inc New York 1956
83 Perutz M F and Mitchison J M State of haemoglobin in sickle-cell anaemia Nature London 166 677 1950

84 Horowitz N H and Fling M Genetic determination of tyrosinase thermostability in Neurospora Genetic 38 360 1953
85 Danneel R Ihr genetik der Kaninchenfärbung Erg bn Biol 18 55 1941
86 Szeinberg A Sheba C and Adam A Selective occurrence of glutathione instability in red blood corpuscles of the various Jewish tribes Blood 13 1043 1958
87 Childs B Zinkham W Browne E A Kimbro E L and Torbert J V A genetic study of a defect in glutathione metabolism of the erythrocyte Bull Johns Hopkins Hosp 102 21 1958
88 Carson P E Flanagan C L Ickes C E and Alving A S Enzymatic deficiency in primaquine-sensitive erythrocytes Science 124 484 1956
89 Carson P E Schrier S L and Alving A S Inactivation of glucose-6-phosphate dehydrogenase in human erythrocytes J Lab Clin Med 48 794 1956
90 Beutler E Dern R J and Alving A S The hemolytic effect of primaquine IV The relationship of cell age to hemolysis J Lab Clin Med 44 439 1954
91 Horsfall W R and Smithies O Genetic control of some human serum β-globulins Science 128 35 1958
92 Giblett E R Hickman C G and Smithies O Serum transferrins Nature London 183 1589 1959
93 Harris H Robson E B and Siniscalco M β Globulin variants in man Nature London 182 452 1958
94 Sutton H E Unpublished results
95 Kabat E A *Blood Group Substances Their Chemistry and Immunochemistry* Academic Press Inc New York 1956
96 Grubb R Agglutination of erythrocytes coated with incomplete anti Rh by certain rheumatoid arthritic sera and some other sera Acta path et microbiol scandinav 34 195 1956
97 Grubb R and Laurell A B Hereditary serological human serum groups Acta path et microbiol scandinav 34 390 1956
98 Goodman M and Campbell D H Differences in antigenic specificity of human normal adult fetal and sickle cell anemia hemoglobin Blood 8 422 1953
99 Bearn A G and Franklin E C Some genetical implications of physical studies of human haptoglobins Science 128 596 1958
100 Spiegelman S and Campbell A M The significance of induced enzyme formation in *Currents in Biochemical Research* 1956 edited by D E Green pp 115 161 Interscience New York 1956
101 Monod J Remarks on the mechanism of enzyme induction in *Enzymes Units of Biological Structure and Function* edited by O H Gaebler pp 7 8 Academic Press Inc New York 1956
102 Novick A and Weiner M Enzyme induction as an all-or none phenomenon Proc Nat Acad Sc (U S) 43 553 1957
103 Knox W E and Mehler A H The adaptive increase of the tryptophan peroxidase-oxidase system of liver Science 113 237 1951
104 Graham J B McLendon W W and Brinkhous K M Mild hemophilia an allelic form of the disease Am J M Sc 225 46 1953
105 Mitoma C Auld R M and Udenfriend S On the nature of enzymatic defect in phenylpyruvic oligophrenia Proc Soc Exper Biol & Med 94 634 1957
106 Anderson E P Kalckar H M and Isselbacher K J Defect in uptake of galactose 1 phosphate into liver nucleotides in congenital galactosemia Science 125 113 1957
107 Kalckar H M Biochemical mutations in man and microorganism Science 125 105 1957
108 Woolf L I Griffiths R and Moncrieff A Treatment of phenylketonuria with a diet low in phenylalanine Brit M J 1 57 1955
109 Holzel A Komrower G M and Wilson V K Aminoaciduria in galactosaemia Brit M J 1 194 1952
110 Hsia D Y Y Driscoll K W Troll W and Knox W E Detection by

phenylalanine tolerance tests of heterozygous carriers of phenylketonuria Nature London 178 1?3) 1956
111 Hsia D Y Y and Driscoll K W Detection of the heterozygous carriers of phenylketonuria Lancet 2 133/ 1956
11? Goppert F Galactosurie nach Milchzuckergabe bei angeborenem familiarem chronischen Leberleiden Berl klin Wchnschr 54 4/3 1917
113 Gruneberg H An analysis of the pleiotropic effects of a new lethal mutation in the rat (Mus norvegicus) Proc Roy Soc London s B 125 123 1938
114 Neel J V and Schull W J Human Heredity University of Chicago Pre Chicago 1951
115 King T J and Briggs R Serial transplantation of embryonic nuclei Col I Spring Harbor Symposia Quant Biol 21 271 1956
116 Stern C Genetic mechanisms in the localized initiation of differentiation Col l Spring Harbor Symposia Quant Biol 21 375 1956
117 Harris H Mittwoch U Robson E B and Warren F L Phenotypes and genotypes in cystinuria Ann Human Genet 20 57 1955
118 Haldane J B S A search for incomplete sex linkage in man Ann Eugenics 7 ?8 1936
119 Wagner R P and Mitchell H K Genetics and Metabolism pp 31–343 Wiley New York 1955
120 Nanney D L The role of cytoplasm in heredity in The Chemical Basis of Heredity edited by W D McElroy and B Glass pp 134–164 Johns Hopkins Press Baltimore 195/
1?1 Sonneborn T M The cytoplasm in heredity Heredity 4 11 1950
12. Mitchell M B and Mitchell H K A case of maternal inheritance in Neurospora crassa Proc Nat Acad Sc (U S) 38 44? 195?

84 Horowitz N H and Fling M Genetic determination of tyrosinase thermostability in Neurospora Genetics 38 360 1953
85 Dannel R Phänogenetik der Kaninchenfärbung Ergebn Biol 18 55 1941
86 Szeinberg A Sheba C and Adam A Selective occurrence of glutathione instability in red blood corpuscles of the various Jewish tribes Blood 13 1043 1958
87 Childs B Zinkham W Browne L A Kimbro E L and Torbert J V A genetic study of a defect in glutathione metabolism of the erythrocyte Bull Johns Hopkins Hosp 102 21 1958
88 Carson P E Flanagan C L Ickes C E and Alving A S Enzymatic deficiency in primaquine-sensitive erythrocytes Science 124 484 1956
89 Carson P E Schrier S L and Alving A S Inactivation of glucose-6-phosphate dehydrogenase in human erythrocytes J Lab Clin Med 48 794 1956
90 Beutler E Dern R J and Alving A S The hemolytic effect of primaquine IV The relationship of cell age to hemolysis J Lab Clin Med 44 439 1954
91 Horsfall W R and Smithies O Genetic control of some human serum β globulins Science 128 35 1958
92 Giblett E R Hickman C G and Smithies O Serum transferrins Nature London 183 1589 1959
93 Harris H Robson E B and Siniscalco M β Globulin variants in man Nature London 182 452 1958
94 Sutton H E Unpublished results
95 Kabat E A *Blood Group Substances Their Chemistry and Immunochemistry* Academic Press Inc New York 1956
96 Grubb R Agglutination of erythrocytes coated with incomplete anti Rh by certain rheumatoid arthritic sera and some other sera Acta path et microbiol scandinav 34 195 1956
97 Grubb R and Laurell A B Hereditary serological human serum groups Acta path et microbiol scandinav 34 390 1956
98 Goodman M and Campbell D H Differences in antigenic specificity of human normal adult fetal and sickle cell anemia hemoglobin Blood 8 4 1953
99 Bearn A G and Franklin E C Some genetical implications of physical studies of human haptoglobins Science 128 596 1958
100 Spiegelman S and Campbell A M The significance of induced enzyme formation in *Currents in Biochemical Research* 1956 edited by D E Green pp 115 161 Interscience New York 1956
101 Monod J Remarks on the mechanism of enzyme induction in *Enzymes Units of Biological Structure and Function* edited by O H Gaebler pp 7 28 Academic Press Inc New York 1956
102 Novick A and Weiner M Enzyme induction as an all-or none phenomenon Proc Nat Acad Sc (U S) 43 553 1957
103 Knox W E and Mehler A H The adaptive increase of the tryptophan peroxidase-oxidase system of liver Science 113 237 1951
104 Graham J B McLendon W W and Brinkhous K M Mild hemophilia an allelic form of the disease Am J M Sc 225 46 1953
105 Mitoma C Auld R M and Udenfriend S On the nature of enzymatic defect in phenylpyruvic oligophrenia Proc Soc Exper Biol & Med 94 634 1957
106 Anderson E P Kalckar H M and Isselbacher K J Defect in uptake of galactose-1 phosphate into liver nucleotides in congenital galactosemia Science 125 113 1957
107 Kalckar H M Biochemical mutations in man and microorganisms Science 125 105 1957
108 Woolf L I Griffiths R and Moncrieff A Treatment of phenylketonuria with a diet low in phenylalanine Brit M J 1 57 1955
109 Holzel A Komrower G M and Wilson V K Aminoaciduria in galactosaemia Brit M J 1 194 1952
110 Hsia D Y Y Driscoll K W Troll W and Knox W E Detection by

phenylalanine tolerance te ts of heterozygous carriers of phenylketonuria Nature London 178 1?39 1956
111 Hsia D Y Y and Dri coll K W Detection of the heterozygous carriers of phenylketonuria Lancet 2 133" 1956
11? Goppert F Galacto uric nach Milchzuckergabe bei angel orenem familiarem chronischen Leberleiden Berl klin Wchn chr 54 473 1917
113 Gruneberg H An analy is of the pleiotropic effects of a ne v lethal mutation in the rat (*Mus norvegicus*) Proc Roy Soc London s B 125 1 3 1938
114 Neel J V and Schull W J *Human Heredity* University of Chicago Pre Chicago 1954
115 King T J and Briggs R Serial tran plantation of embryonic nuclei Col l Spring Harbor Sympo ia Quant Biol 21 ?/1 1956
116 Stern C Genetic mechanisms in the localize l initiation of differentiation Col l Spring Harbor Symposia Quant Biol 21 3/5 1956
117 Harris H Mittwoch U Robson E B and Warren F L Phenotypes and genotypes in cystinuria Ann Human Genet 20 57 1955
118 Haldane J B S A search for incomplete sex linkage in man Ann Eugenics 7 28 1936
119 Wagner R P and Mitchell H K *Genetics and Metabolism* pp 312–343 Wiley New York 1955
1?0 Nanney D L The role of cytoplasm in heredity in *The Ch mical Ba is of Heredity* edited by W D McElroy and B Glass pp 134–164 Johns Hopkins Press Baltimore 1957
121 Sonneborn T M The cytoplasm in heredity Heredity 4 11 1950
1?2 Mitchell M B and Mitchell H K A case of maternal inheritance in *Neu ospora cra sa* Proc Nat Acad Sc (U S) 38 44? 195?

84 Horowitz N H, and Fling M Genetic determination of tyrosinase thermostability in Neurospora. Genetics 38 360 1953
85 Dannenberg H Die Hämogenetik der Kaninchenfärbung Erg Biol 18 5 1941
86 Szeinberg A Sheba C, and Adam A Selective occurrence of glutathione instability in red blood corpuscles of the various Jewish tribes Blood 13 1043 1958
87 Childs B, Zinkham W Browne E A Kimbro E L and Torbert J V A genetic study of a defect in glutathione metabolism of the erythrocyte Bull Johns Hopkins Hosp 102 21 1958
88 Carson P E, Flanagan C L, Ickes C E and Alving A S Enzymatic deficiency in primaquine-sensitive erythrocytes Science 124 484 1956
89 Carson P E, Schrier S L and Alving A S Inactivation of glucose-6-phosphate dehydrogenase in human erythrocytes J Lab Clin Med 48 791 1956
90 Beutler E, Dern R J and Alving A S The hemolytic effect of primaquine IV The relationship of cell age to hemolysis J Lab Clin Med 44 439 1954
91 Horsfall W L and Smithies O Genetic control of some human serum β-globulins Science 128 35 1958
92 Giblett E R, Hickman C G and Smithies O Serum transferrins Nature London 183 1589 1959
93 Harris H Robson E B and Siniscalco M β-Globulin variants in man Nature London 182 452 1958
94 Sutton H E Unpublished results
95 Kabat E A Blood Group Substances Their Chemistry and Immunochemistry Academic Press Inc, New York 1956
96 Grubb R Agglutination of erythrocytes coated with incomplete anti Rh by certain rheumatoid arthritic sera and some other sera Acta path et microbiol scandinav 34 13 1956
97 Grubb R, and Laurell A B Hereditary serological human serum groups Acta path et microbiol scandinav 34 390 1956
98 Goodman M and Campbell D H Differences in antigenic specificity of human normal adult, fetal and sickle cell anemia hemoglobin Blood 8 422 1953
99 Bearn A G and Franklin E C Some genetical implications of physical studies of human haptoglobins Science 128 596 1958
100 Spiegelman S and Campbell A M The significance of induced enzyme formation in Currents in Biochemical Research edited by D E Green pp 115-161 Interscience New York 1956
101 Monod J Remarks on the mechanism of enzyme induction in Enzymes Units of Biological Structure and Function edited by O H Gaebler pp 7-28 Academic Press Inc New York 1956
102 Novick A and Weiner M Enzyme induction as an all-or-none phenomenon Proc Nat Acad Sc (U S) 43 553 1957
103 Knox W E and Mehler A H The adaptive increase of the tryptophan peroxidase-oxidase system of liver Science 113 237 1951
104 Graham J B McLendon W W and Brinkhous K M Mild hemophilia an allelic form of the disease Am J M Sc 225 46 1953
105 Mitoma C Auld R M and Udenfriend S On the nature of enzymatic defect in phenylpyruvic oligophrenia Proc Soc Exper Biol & Med 94 634 1957
106 Anderson E P Kalckar H M and Isselbacher K J Defect in uptake of galactose 1-phosphate into liver nucleotides in congenital galactosemia Science 125 113 1957
107 Kalckar H M Biochemical mutations in man and microorganism Science 125 105 1957
108 Woolf L I Griffiths R and Moncrieff A Treatment of phenylketonuria with a diet low in phenylalanine Brit M J 1 57 1955
109 Holzel A Komrower G M and Wilson V K Aminoaciduria in galactosaemia Brit M J 1 194 1952
110 Hsia D Y Y Driscoll K W Troll W and Knox W E Detection by

Part Two

# Diseases Manifest Primarily as Disorders of Carbohydrate Metabolism

## Chapter 3

# Diabetes Mellitus*

*Albert L. Renold and George F. Cahill, Jr.*

Although there is little reason to doubt that future developments will fully justify the decision of the editors to consider diabetes mellitus an inborn error of metabolism, there is equally little reason to deny that the nature of this error is, at present, shrouded in darkness. Why, then, discuss this ancient disorder in a text whose title implies some degree of knowledge about the nature of the inherited metabolic aberrations to be discussed? Justification may perhaps be derived from at least three considerations.

1. Diabetes mellitus is a widespread and complex disorder of metabolism intimately related in history to the development of understanding of the metabolism of carbohydrate, protein, and fat, and thus well suited for the illustration of over-all schemes of intermediary metabolism and of their interrelations. Its historical importance is compounded by its practical importance, which derives from its high level of incidence and its prevalence among all human races and societies.

2. Diabetes mellitus—more precisely, the susceptibility to diabetes mellitus—is indeed conditioned by genetic factors and may thus quite properly be termed inborn.

3. A discussion of the knowledge accumulated about this disorder may serve to illustrate how much ingenious and exhaustive effort can be devoted to the study of a metabolic disorder without even approaching the nature of the inborn disorder. This last consideration is possibly more important than would first appear, since it may be useful in tempering excessive enthusiasm with regard to the final nature of the inborn metabolic errors to be described in other sections of this volume. Although without question *closer* to the primary defect, some of these may also

* The senior author would like to express his appreciation to the Allen Foundation, Inc., Rye, N.Y., for the support of his activities at the Baker Clinic Research Laboratory, New England Deaconess Hospital.

of moderate hyperglycemia alone The causal relationships assumed in the sequence of events just described are convenient and likely but in many aspects still hypothetical More will be said on this subject below during consideration of the metabolic effects of insulin

*Chronic*

The term *chronic diabetic syndrome* refers to the many clinical manifestations which in addition to some or all of the manifestations of the acute diabetic syndrome are associated with the presence of diabetes of long duration These manifestations may affect any system or organ although the organs most frequently affected are the kidneys eyes heart muscle and portions of the nervous system including the peripheral nerves A large part of these manifestations appears to have a vascular basis sometimes interpreted as accelerated vascular aging although more specific alterations of capillaries arterioles and venules are frequently present particularly in the eyes and kidneys The frequent presence of abnormal deposits of glycogen and mucopolysaccharides in diabetic cells in general and in the basement membrane of capillaries and small blood vessels in particular has led to the suggestion that a metabolic aberration of small vessel walls might be at the origin of many pathologic and clinical manifestations of the chronic diabetic syndrome [6–8] Whether the acute and the chronic diabetic syndromes are directly related causally is as yet uncertain although likely in the opinion of many

Most of the following discussion will be limited to the acute diabetic syndrome Whether or not the manifestations of the chronic syndrome are related to an inborn error of metabolism and if so whether or not the error is that responsible for the acute syndrome are as yet so hypothetic as to preclude useful discussion in this text The clinical differentiation of two or more diabetic syndromes (e g growth-onset versus adult diabetes) will be briefly discussed below under Insulin

## GENETIC CONSIDERATIONS

*Incidence*

The over all incidence of diabetes mellitus is difficult to ascertain since the disease may declare itself late in life and may go unrecognized for many years The most accurate attempt to evaluate this incidence stems from the studies of Wilkerson and Krall who in 1947 tested 3 516 of 4 983 inhabitants of a New England community with an age distribution closely approximating that of the United States as a whole [9] They found 40 known and 30 previously unknown diabetic patients i e a prevalence of 2 0 per cent of those tested or a projected 1 7 per cent of the entire population of the town Follow up studies indicate that the

prove to be but *manifestations* of the primary defect separated from it by one or several intermediate metabolic or structural conductors

Published evidence concerning every aspect of diabetes mellitus is so extensive as to preclude any attempt to provide complete coverage of possible topics, or of any single topic Accordingly the authors have, *volens nolens*, exercised arbitrary selectiveness with emphasis upon concepts and upon attempted synthesis and organization rather than upon erudition and documentation Major omissions will be noted by all and these the authors regret although without apology

Rather than providing an abbreviated historical introduction, the authors refer to the remarkable monographs of Allen [*1 2*] for the pre-insulin period and to the monograph of Jensen [*3*] for the period which led to the discovery and application of insulin

## DIABETIC SYNDROMES

Although Dobson [*4*] concluded from his experiments published in 1776 that the saccharine matter secreted by the kidney in diabetes was "not formed by the secretory organ but previously existed in the serum of the blood,' Ambrosiani is credited with having first recorded in 1835 that the blood of diabetic patients contains more sugar than that of nondiabetic individuals [*3*] and thus with having established what is now accepted as the only essential sign of the disorder i e *hyperglycemia* The severity and duration of hyperglycemia (with or without provocation) required to establish the diagnosis of the disease vary from medical center to medical center a widely accepted set of criteria is that established by Joslin and his group [*5*]

### *Acute*

Under the term *acute diabetic syndrome* are grouped all those manifestations more or less readily explained as derived from the cardinal manifestation—hyperglycemia With increasing hyperglycemia, after the renal tubular capacity for glucose reabsorption is exceeded glycosuria and with it, polyuria result Loss of water and glucose leads to increased thirst and hunger Glucose loss by draining the carbohydrate supplies of the organism results in increased need for mobilization and catabolism of proteins and fats This results in weight loss and when the mobilization of fats becomes excessive in the accumulation of ketone bodies in the blood and urine, with ketonuria and ketonemia Since the ketone bodies are primarily anionic cations accompany their urinary excretion When the blood levels of ketone bodies become excessive ketoacidosis and by as yet unclear mechanisms coma and death result The sequence is frequently rather rapid (several hours to several days) although it may remain transiently or permanently arrested at any stage even at that

*Inheritance Pattern*

The opinion most prevalent today assigns to the predisposition for diabetes mellitus a simple Mendelian recessive mode of inheritance [20] ✓ This is first and rather directly suggested by the tendency for the disorder to skip generations a repeatedly documented observation accessible to any thoughtful questioner Second many comparisons between observed and predicted incidence of diabetes in specific areas or groups have yielded best agreement when the predicted figures were based on the assumption of a simple Mendelian type of inheritance Furthermore a number of very extensive and well-documented genealogies can be interpreted on this basis only In the population group studied by Hanhart and referred to above [16] the incidence of diabetes among the 1 623 siblings of the 304 diabetic subjects was 27 3 per cent whereas it was 22 0 per cent in their 646 offsprings both figures being corrected statistically for the age behavior of diabetes There was thus a significantly greater incidence of diabetes among the siblings than among the children of diabetic persons a pattern which can be explained only on the basis of recessive inheritance The significance of this observation is further increased by comparing this incidence with that found among 114 children aged 40 or more of 35 instances of conjugal diabetes (both parents having known diabetes) also studied by Hanhart [15] and drawn from the same general population When corrected for the age behavior of diabetes the incidence obtained in this group was 94 per cent!

The arguments brought forward in the preceding paragraph strongly suggest the *participation* of a simple Mendelian recessive trait in the inheritance of the potentiality for developing diabetes they in no way however establish a *monopoly* for such a trait Genealogies singly or in small numbers strongly suggesting simple dominant [21 22] or sex linked recessive inheritance [23] have been reported The occurrence of diabetes in three successive generations is not really rare while its occurrence in four successive generations has been recorded twice in the experience of the Joslin Clinic [13] However it is evident that the combination of a very high incidence of diabetes at least in certain areas (see first paragraph) with the prolonged latency characteristic of this disorder may lead to any number of freak genealogies resulting from inability to recognize either the heterozygous carrier or frequently the homozygous latent diabetic In many instances the *pseudodominant* nature of what first appeared to be a dominant pattern has been established by extension of the genealogy or by the passage of time

*Penetrance*

In order to explain the absence of diabetes in a significant percentage of identical twin mates of diabetic twins (Table 3 1) as well as the fact that

incidence of the disease in the original population if followed to old age or death will be at the very least double that of the prevalence initially found i e, 3 5 per cent or more likely higher [10, 11] Although these figures are higher than those generally accepted [11a] this may primarily be the result of the adequacy of the procedures used for the detection of abnormal glucose metabolism in this study These figures are mentioned here since acceptance of a high rate of incidence of diabetes mellitus (or rather of the tendency to develop the disorder) is necessary in order to explain some of the genetic irregularities to be discussed below

*Evidence for Hereditary Nature of Diabetic Potential*

That the potentiality to develop diabetes mellitus is inherited has been surmised for many centuries [12] and is now an established fact This rests primarily upon (1) the greater frequency of diabetic persons among close relatives of known diabetic patients than among suitable control populations and (2) the even more frequent occurrence of diabetes in

TABLE 3 1 INCIDENCE OF DIABETES IN TWO SERIES OF TWINS

| Author | Similar twins | | | Dissimilar twins | | |
|---|---|---|---|---|---|---|
| | Total No | Diabetes present in both | | Total No | Diabetes present in both | |
| | | No | Per cent | | No | Per cent |
| Then Bergh | 16 | 30 | 65 | 87 | 18 | 22 |
| White | 33 | 16 | 48 | 63 | 2 | 3 |
| Total | 79 | 46 | 58 | 150 | 20 | 13 |

the mates of similar diabetic twins With regard to the first point only one study will be mentioned here among many [13] Hanhart [14–16] has analyzed 304 genealogies of diabetic patients of the Joslin Clinic with 18 493 members and compared them with 110 genealogies of nondiabetic individuals with 6 042 members Both groups were drawn from a socially and racially similar population Among the 18 493 members of the diabetic genealogies 975 diabetic persons were found an incidence of 5 3 per cent whereas only 74 diabetic persons or 1 2 per cent were found among the 6 042 members of the nondiabetic families The incidence of diabetes in two series of twins [17 18] is shown in Table 3 1 Differences between the two series are primarily the result of the criteria used for establishing presence or absence of the disorder in the twin mates of diabetic twins It is difficult to avoid the conclusion however that whatever the true incidence the incidence of the disease in identical twin mates grossly exceeds the incidence which may be expected in *any* general population group

of the metabolic decompensation process accompanying pregnancy may become permanent [32] The reported increased incidence of diabetes in multiparous women over 40 years of age (when compared with men or with women not having borne children) supports this contention [35] This relation between parity and the development of diabetes after the age of 40 however, is not universally accepted [26]

*Etiologically Distinct Forms of Diabetes*

The variable clinical manifestations of diabetes mellitus in man have frequently led to the postulate of etiologically distinct forms of the dis order This concept would be strengthened were it possible to demon strate at least some degree of genetic separation of these clinically dis tinct syndromes Generally it is accepted today that such a genetic separation has not been demonstrated [5, 16 20 36 37] and that, for instance growth-onset type and adult onset type diabetes frequently occur within the same family A few opinions to the contrary have been recorded [38 39] The widespread prevalence of the diabetic trait (or traits) in the general population greatly adds to the difficulty of establish ing the presence or absence of genetic separation of different diabetic syndromes particularly in highly mobile and mixed population groups

It is of interest therefore to find that although there is a slightly higher incidence of diabetes among Jews and although it has been re peatedly stated that this might be the result of the somewhat greater degree of inbreeding of this population diabetes mellitus among Jews does not appear to have assumed any characteristic clinical pattern

*Antecedence and Anticipation*

The terms antecedence and anticipation in the inheritance of diabetes mellitus were used to describe the tendency for the overt disease to occur earlier in life in succeeding generations Fortunately this concept for which it was difficult to find a reasonable genetic explanation has been shown to represent most probably a statistic rather than genetic phenomenon [40] it should dissolve with the passage of time and the recording of genealogies with adequate follow up periods

At the beginning of this section it was stated that the most intensive study designed to estimate the true incidence of diabetes in the United States indicated a value of 3 5 per cent or more Assuming that indi viduals liable to diabetes are homozygous for a recessive gene (d) and basing their estimate upon two different sets of data from this country Steinberg and Wilder [20 36 37 41 42] concluded that approximately 5 per cent of the population of the United States are homozygous (dd) for the gene determining susceptibility to diabetes These estimates clearly indicate the enormous practical importance of this disorder

the incidence of diabetes among the offspring of marriages between diabetic patients usually lies far below 100 per cent and frequently remains below 100 per cent even when correction for the age behavior of diabetes is made it is customary to state that *the penetrance* (i e, likelihood that a gene will receive its morphologic or functional expression) *of the diabetic trait is incomplete and furthermore variable* This incomplete penetrance could result from the dependance for expression upon either associated genetic factors or associated environmental factors Since the genetic capital of identical twins should be identical the importance of associated environmental factors appears more probable [*19 20*] Furthermore Hanhart [*15*] has studied seven genealogies of conjugal diabetes in which considerable and long standing inbreeding existed thereby increasing the likelihood of the genetic homogeneity of the diabetic trait In all seven families at least one child was diabetic but in no instance were all of them, again suggesting that the variable penetrance of the trait is due to nongenetic factors It is of interest to note that this type of evidence rather clearly suggesting the importance of environmental factors upon the incidence of the disorder within genetically equally jeopardized offsprings offers perhaps the most hopeful basis for eventual prevention of at least overt diabetes

*Pregnancy*

Among the environmental factors which have been considered particular importance may attach to the *environment of the fetus in utero* Since diabetic mothers have been enabled to carry pregnancies to term only relatively recently statistically fully adequate comparisons of the incidence of diabetes in the offsprings of diabetic mothers and in the offsprings of diabetic fathers have not as yet been made a greater early incidence in the former has been suggested [*24 25*] but also questioned [*26*] while the incidence of large babies is very likely greater when the mother was the diabetic or prediabetic partner [*27*] Even mild and unsuspected diabetes during pregnancy (see Prediabetic State below) greatly increases fetal wastage and the pathologic anatomic aberrations found include an abnormal appearance of the fetal islets of Langerhans suggesting hypertrophy [*28 29*] Accordingly it would not be surprising if time were to substantiate the claim that the metabolic state of the mother during gestation may influence the incidence of overt diabetes in the offspring or at least the timing of its onset Experimental models in animals have been suggested [*30, 31*] and may well lead to an earlier answer to this important question

Whereas maternal metabolism must be of importance to the fetus it is also well established that the metabolic changes of *pregnancy* (for the mother) frequently uncover the existence of a previously latent diabetic trait [*32–34*] It has been strongly suggested that the acceleration

diagnosis can be made by methods of testing accepted at the time of their performance With increasing ability to detect the homozygous (dd) carrier of the diabetic trait prediabetes should become an even shorter period of the life of each diabetic patient This is desirable since in the majority of instances early recognition of the presence of homozygocity for diabetes will enable the physician to study and perhaps to influence that important and often extensive early period of the disease which is not accompanied by symptoms but which undoubtedly plays a major role in the timing and nature of its clinical course

In order to detect an even greater number of homozygotes at an even earlier time in their life tests have been devised which study the adequacy of gluco e metabolism under conditions of stress This stress may be the administration of a glucose load or it may combine glucose loading with other stresses such as the administration of cortisone or the coexistence of pregnancy To date the most extensive and successful effort made to detect a large proportion of prediabetic individuals in a given population has been the study by Conn [51 51a] of the relatives of diabetic patients followed by his unit at the University of Michigan Among 387 non diabetic close relatives of known diabetics performance of glucose tolerance tests revealed 71 hitherto unknown diabetics (18 per cent) and performance of a combined cortisone glucose tolerance test in 259 of the subjects of this group which had shown a normal response to glucose alone uncovered another 64 individuals whose response was abnormal by the criteria used (16 per cent of the original group) In contrast an abnormal response to glucose alone was observed in only 1 and to cortisone and glucose in 3 of 125 individuals without *known* diabetic relatives (1 and 3 per cent respectively) Thus in this study testing of 387 close relatives of known diabetics all previously considered unaffected demonstrated that upward of 34 per cent should be considered diabetic by the criteria used

Other investigators [24 32 33] among whom Hoet is particularly active have suggested that advantage should be taken of the physiologic stress of pregnancy to detect the presence of prediabetes in mothers The detection of prediabetic persons in this group may be of special importance both to the mother and to the child and careful therapy of any degree of carbohydrate intolerance during pregnancy has been strongly recommended In the absence of glucose tolerance studies during pregnancy the repeated observation of unusually large babies and particularly progressive increase in infant weight during successive pregnancies suggests maternal prediabetes [52]

Other stressful situations frequently associated with decreased carbohydrate tolerance are puberty obesity and infection Since decreased carbohydrate tolerance in these situations is often temporary only its documentation is of great importance in order to establish prediabetes The frequent association of decreased carbohydrate tolerance and obesity

particularly since the introduction of effective therapy is likely to result in a further increase of this incidence

*Hereditary Diabetes Mellitus in Animals*

Although the occurrence of spontaneous syndromes resembling human acute diabetes mellitus has been noted in many species the authors are aware of the existence of only two fully documented hereditary diabetic syndromes in animals In 1949 Ingalls et al [43] reported the occurrence of a mutation in *mice* of the Jackson Memorial Laboratory Bar Harbor Maine, a mutation resulting in the simultaneous appearance of *obesity and hyperglycemia* The trait is recessive both obesity and hyperglycemia appear to be linked to a single gene The pancreatic islets of these mice are hypertrophic and increased insulin like activity has been found both in pancreatic extracts [44] and in blood serum [45] of the obese-hyperglycemic animals when compared with normoglycemic nonobese litter mates The affected mice do not develop ketosis [46] In 1959, Meier and Yerganian reported occurrence of diabetes mellitus *during inbreeding of several lines of Chinese hamsters (Cricetulus griseus)* [47 48] In contrast to the obese-hyperglycemic mice diabetic Chinese hamsters exhibit degenerative changes of the islets of Langerhans and tendency to profuse glucosuria and ketoacidosis Liver slices from diabetic animals demonstrate a metabolic derangement quite comparable to that seen in rats with severe alloxan diabetes [49] The possible occurrence of some features of the chronic diabetic syndrome in these animals has been reported in preliminary form [48] If laboratory animals with hereditary forms of diabetes mellitus should become available it is evident that this might well be one of the most significant forward steps in understanding the genetic and environmental factors concerned with diabetes mellitus in man

It may be of interest to recall at this point that sensitivity to insulin is also subject to genetic control Not only do different species vary greatly with regard to insulin responsiveness but within a given species unusually sensitive and unusually resistant strains have been described Thus the KL strain of mice may be given intraperitoneally from 300 to 500 units of insulin with impunity whereas their controls regularly convulse and succumb after the injection of doses well below 50 units [50]

*Prediabetic State*

If the hereditary nature of diabetes mellitus is accepted and since it is known that the overt disease may not develop until late in life it is evident that a large number of individuals who are genetically destined to become diabetic are considered to be totally unaffected for significant periods of their life As defined by Conn and Fajans [51 51a] the "prediabetic state" must be regarded as existing in an individual destined to become diabetic from the time of conception to the time when a definitive

guinea pigs and has recently been confirmed by Wright [58] in rabbits injected with antibodies to beef insulin obtained in guinea pigs (Fig 3 1) As would be anticipated this type of diabetes is reversible with discontinuation of the administration of these species specific insulin antibodies

It would seem almost impossible to produce a better biologic correlation between the experimental production of the diabetic syndrome on the one hand and interference with the endocrine function of the pancreas on the other If in addition one considers that the administration of insulin is capable of completely reversing all known features of the acute diabetic syndrome the weight of evidence seems almost overwhelming There are however, additional experimental diabetic syndromes in animals in which the relationship to the insulin producing cells of the pancreas is less evident Among these are (4) the prolonged administration of anterior pituitary extracts [59] or of purified pituitary growth hormone [60] preparations as well as in suitably prepared animals of certain adrenocortical [61] and thyroid hormones [62] It has been postulated that the metabolic effects of these hormones create conditions leading to persistently increased insulin secretion [63] and thereby to eventual inadequacy of the insulin secreting cells A similar mechanism has been postulated for the production of a diabetic syndrome by (5) the prolonged administration of glucose in quantities sufficient to maintain a greatly elevated blood glucose level throughout the greater portion of the 24 hr period [64] This type of experimental diabetes has been reproducibly obtained in only one species the cat

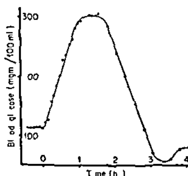



Fig 3 1 Concentration of glucose in the blood of a conscious female rabbit (? 65 kg) following the intravenous injection at 0 time of serum (2 7 ml) from guinea pigs sensitized to bovine insulin (From Wright [58])

*Human Diabetes*

Human diabetes mellitus has been produced by pancreatectomy incident to removal of malignant tumors and has been noted during and after pancreatitis In addition hemochromatosis which is accompanied by a deposition of iron pigment in the pancreas with secondary fibrosis and loss of functioning tissue including particularly islet cells is a well established cause of diabetes mellitus In these instances all relatively rare some correlation exists between the development of diabetes and destruction of the pancreatic islets In the great majority of cases of spontaneous diabetes mellitus in man however the correlation between

has sometimes been ascribed to a genetic rather than environmental link It is tempting to believe that this may be true in certain instances (particularly when one considers the existence of the obese hyperglycemic syndrome in mice!), but at present it does not seem feasible to dissociate genetic and environmental factors in any of the studies reported

The great importance of prediabetes and of developing methods suitable for its detection is based on at least three major considerations (1) ability to detect homozygotes (dd) for the diabetic trait would place genetic studies of diabetes on a sounder basis, thereby opening the way for sounder genetic advice, wherever acceptable (2) it would seem evidently desirable to gain insight into the *true* incidence of the diabetic trait in order to uncover some of the interrelations between diabetes and the several major disorders of cardiovascular renal origin which afflict civilized man, (3) most importantly if large groups of individuals were known to be the as yet asymptomatic carriers of the homozygous trait investigative programs designed to establish some of the environmental conditions needed to precipitate or prevent the onset of the overt phase of the disorder could be conceived with at least some degree of intelligence and chance of success

## ENDOCRINE DEFECTS IN DIABETES

### *Experimental Diabetes*

The major endocrine defect in diabetes mellitus is considered to be absolute or relative insulin deficiency It should be pointed out at the outset that this does not necessarily imply a primarily defective insulin producing tissue

Understanding of the proper nature of the major endocrine defect is primarily based upon observations concerning the production of *experimental diabetes* and goes back to the demonstration by von Mehring✓ and Minkowsky in 1889 that removal of the pancreas in dogs produces a syndrome grossly similar to the acute diabetic syndrome in man [53] As of the present experimental diabetes mellitus can be produced by one of the following procedures (1) the surgical removal of all or at least a large portion of the pancreas (2) the chemical destruction of all or at least a large portion of the beta cells of the islets of Langerhans a destruction which can be achieved with substances such as alloxan [54] dehydroascorbic acid [55] or dithizone [56] which in appropriate doses produce a selective necrosis of the beta cells (3) the inactivation of circulating insulin by the administration to animals of one species of antibodies effective against its own insulin and obtained by immunizing other animals This type of diabetic syndrome was first described by Moloney [57] in mice injected with antibodies to pig insulin obtained in

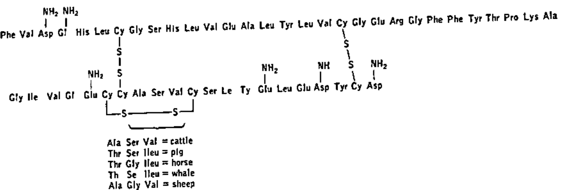



Fig 3-. Chemical formula of insulin (*Modified from Sanger [68]*)

morphologic damage to the islets and the development and severity of the disease is not good, but it is not so poor as has been frequently stated

Whereas it is true as recently restated by Warren and LeCompte [8] that from one-fourth to one-half of all cases of diabetes show no changes √ in the pancreas extensive enough to constitute a convincing cause of insulin deficiency these authors also point out that this is true only when one considers the material obtained and *studied histologically by routine methods* When special attention is paid to the insulin producing beta cells and to the tedious and time-consuming estimation of total mass of pancreatic beta cells, a decreased volume or abnormal appearance√ of the beta cells is almost uniformly found [65 66] This anomaly is most marked in patients with growth onset type of diabetes in whom the acute diabetic syndrome if untreated leads to severe ketoacidosis it is less marked in the adult frequently obese type of diabetic without a tendency to ketoacidosis These pathologic findings are in good agreement with the estimates of pancreatic insulin extractible at autopsy which will be referred to below [67]

In the opinion of the authors it would seem quite well established that in human diabetes as in experimental diabetes there exists some degree of anomaly of the insulin producing beta cells of the islets of Langerhans in a majority of instances This statement however in no way precludes the possible frequent participation of additional etiologic factors nor does it necessarily imply acceptance of the primary nature of the pancreatic defect Although many interesting observations have been made with regard to the possible etiologic participation of other endocrine glands in diabetes mellitus and although their participation in the clinical manifestations of the disorder is unquestioned, this topic will not be discussed here since it raises too many purely hypothetic questions and does not lend itself to reasonably succinct discussion from the major point of view of this volume i e from the point of view of the nature of the inherited defect That a diabetic syndrome is frequently associated with *overt* dysfunctions of pituitary and/or adrenal glands is generally accepted

## INSULIN

### *Chemistry*

The detailed analysis of the structure of insulin undoubtedly one of the major accomplishments of modern analytic protein chemistry [68] has led to the formula shown in Fig 3 2 Insulin is a relatively small protein consisting of two polypeptide chains the shorter A chain and the longer B chain The A chain is made up of 21 amino acid residues and the B chain of 30 residues The two chains are linked by two disulfide bridges and there exists in addition a third internal disulfide bridge within the

and all of them result not only in increased molecular weight but also in markedly altered solubility characteristics In the presence of zinc ions previously soluble insulin becomes insoluble and insulin precipitates in crystalline form Heating of relatively concentrated insulin solutions at low pH results in the formation of insulin fibrils and spherites a phenomenon quite characteristic for insulin Some of these relationships are illustrated in Fig 3 3 redrawn from Sanger's publication [69 70] each hollow sphere represents a *double* insulin molecule of molecular weight 12 000

The shape of the insulin molecule in aqueous solution appears to be roughly spherical Whether this is true in complex solutions such as plasma is unknown The detailed nature of the spatial arrangement of the polypeptide chains of insulin is still the subject of controversy This spatial arrangement may not be intimately related to the biologic activity of insulin since it has been reported that irreversible stretching of the chains does not grossly modify the biologic activity of the hormone [71]

*Biosynthesis*

As yet nothing is known about the biochemical mechanisms leading to the synthesis of insulin by the beta cells of the islets of Langerhans Accordingly nothing is known about the presence or absence of abnormalities or deficiencies of these mechanisms in diabetes

*Storage*

The quantities of insulin which may be extracted from the pancreas vary from species to species [72] in normal man the order of magnitude is 4 units per gm of pancreas [67 73] Accordingly it may be assumed that upwards of 200 units of insulin (i e about 10 mg of the pure hormone) is not infrequently stored in the beta cells of the islets of Langerhans of any given individual This amount of insulin is potentially dangerous and it would seem evident that it must be stored in a form precluding sudden release As already discussed the insulin molecule per se is freely soluble in water at the pH presumed to exist within cells which is quite alkaline with relation to the isoelectric point of the hormone It is likely therefore that a major part of the insulin contained in beta cells is present in a modified state resulting in its decreased solubility Working with the giant islets of the flounder Maske [74] has indeed shown this to be the case since after homogenization of the islet cells insulin activity was persistently associated with centrifugable fractions and could not be eluted therefrom

It is of great interest that two of the mechanisms known to decrease the solubility of pure insulin (depicted in Fig 3 3) could easily be operative in the intact beta cells of the islets of Langerhans First it has been

A chain That portion of the A chain which lies between the cystine residues forming the disulfide bridges is of special interest since it appears to be the main (possibly the only) site of molecular differentiation between species As also indicated in the figure insulins obtained from cattle, pig sheep horse and whale differ from one another only by the nature and the arrangements of the three amino acid residues contained within this disulfide ring Pig and whale insulin are identical Thus although this part of the molecule clearly is not essential for the biologic activity of the hormone, it is likely to be involved in its immunologic behavior [1]

The unit described in Fig 3 2 is the smallest known carrier of biologic insulin activity Its molecular weight lies somewhat below 6 000 and its

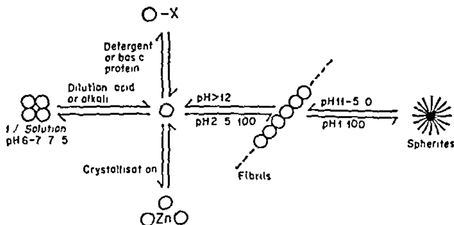



Fig 3-3 Molecular associations of insulin Each hollow sphere represents an insulin dimer of molecular weight 12 000 (*Redrawn from Sanger [63]*)

isoelectric point between pH 5 3 and 5 4 Whereas insulin is almost insoluble in water at this pH (1 100 000) this solubility increases rapidly if the pH is shifted even slightly upward or downward In solution insulin molecules undergo varying degrees of aggregation and accordingly, the true molecular weight observed under a given set of conditions varies by units of 6,000 Under most conditions it would appear that at least two molecules of insulin aggregate and that further aggregation occurs by units of molecular weight 12 000 Of particular interest is the tendency of insulin to react with divalent cations particularly zinc ions with basic proteins such as protamines or histones or with itself under certain conditions These reactions are reversible

[1] Recent work from Sanger s laboratory (D S Nichol and L F Smith personal communication) indicates a further area of the insulin molecule which appears to vary between species Human insulin is identical with pig insulin except for the C-terminal amino acid residue of the B chain aspartic acid having replaced alanine In rabbit insulin the C-terminal residue of the B chain is serine

alter the histologic appearance and in some instances the extractable insulin content of the pancreatic islets [77a] the only fully established stimulus to insulin secretion is the presence of an elevated level of blood glucose [78–81] and probably of other sugars as well Accordingly it is to be anticipated that satisfactory hypotheses for the mechanism of insulin release will include possible mechanisms relating increased availability of glucose to accelerated release of the hormone

An interesting mechanism has been suggested by Maske [74] linking insulin release to the interaction of stored insulin with zinc It is known that substances which easily chelate with zinc are capable of releasing insulin from the zinc insulin complex Among these substances are many intermediary products of cell metabolism such as citrate oxalacetate, and glutathione Stimulation of glucose metabolism within the islet cells may result in accumulation of one of these substances thus inducing insulin solubilization and release On the other hand release could be effected by splitting insulin from its protein carrier or carriers as has been suggested for protamine zinc insulin [82] Enzymes capable of effecting this separation have been described in tissues and in blood

It should also be mentioned that a most intriguing peculiarity of beta cell metabolism has been recently described It has been demonstrated that these cells contain appreciable concentrations of glucose 6-phosphatase an enzyme previously considered limited to liver kidney and intestinal mucosa [83]! It is conceivable that the activity of glucose metabolism within these cells could be in part controlled by altering the rate of glucose 6-phosphate hydrolysis relative to the rate of glucose 6-phosphate metabolism by other pathways Although not universally accepted as yet accumulating evidence indicates that the hypoglycemic sulfonylureas bring about an accelerated release of insulin from preformed stores [84] In this instance accumulation of citrate [85] perhaps on the basis of accelerated glucose 6-phosphate metabolism secondary to glucose 6 phosphatase inhibition [84] has been suggested as a possible mechanism

The present state of knowledge of insulin storage and release in human diabetes mellitus will be outlined below

*Transport*

After insulin has been released it is transported in the blood stream to sites of action within the body Since proteins frequently interact it is not surprising to find that insulin appears to undergo several types of interactions with protein components of blood A major portion of insulin activity or of insulin labeled with $I^{131}$ added to serum separates electrophoretically into the $\beta$ fraction [86 87] or by selective precipitation into fractions II and III [87–89] using Cohn s nomenclature A quite specific type of interaction has been suggested by Antoniades [90] i e

established that these cells contain an unusually high concentration of zinc [74 75] Second it is also known that they contain several small molecular basic proteins Indeed, Lindner [76] suggested some years ago that when insulin is extracted from fresh beef pancreas with relatively mild techniques the product obtained is insulin associated with a small molecular basic protein probably a histone Insulin so obtained has been called ' Nativinsulin and has been employed as a long acting form of insulin therapy just prior to the introduction of protamine zinc insulin by Hagedorn The pharmacologic problem of artificially producing an injectable insulin which would not be rapidly soluble and absorbed from the injection site was solved by selecting protamine zinc insulin which combined the ability of both zinc and protamine (a small molecular basic protein) to produce the desired change in the solubility of the hormone!

Histologically, the beta cells of the islets of Langerhans are characterized by granules with rather specific staining properties [8] The insulin content of any given pancreas has been shown in several studies to correlate rather well with its content in beta granules [77] as well as with its islet mass [77a] These granules are almost certainly not insulin per se but they may be insulin binding or insulin storing structures. Since zinc also is present in the beta cells at least in part in granular form the possibility that the beta granules represent zinc protein cores has been raised When relatively pure giant flounder islets were fractionated by Maske [74] the major part of zinc and insulin appeared to be associated with a mitochondrial fraction as demonstrated histologically and also by their further association with succinic dehydrogenase a mitochondrial enzyme

Thus in the beta cells of the islets of Langerhans a complex mechanism has been set up which primarily enables these cells to store appreciable amounts of insulin This mechanism appears to involve the reaction of insulin with zinc and with a basic protein and may be closely associated with the site of synthesis of the hormone It has been suggested that the appearance of the Golgi apparatus may represent a further morphologic index of the activity of insulin secretion [77b]

### *Secretion and Release*

Increased secretion of insulin could be the result either of increased synthesis with immediate release of the product or of increased release of preformed, stored insulin In view of the evidence indicating the presence of significant amounts of stored insulin as well as variation in this amount under different conditions it seems probable that insulin secretion is controlled by mechanisms regulating its release from intracellular stores This release is likely to involve solubilization Although many factors (including hormonal nutritional and neural ones) are known to

*Insulin in Human Diabetes Mellitus*

The discussion on diabetes mellitus presented in this chapter is built on the assumption that the major endocrine defect in diabetes is absolute or relative insulin deficiency The preceeding section on insulin has emphasized the complex nature of the insulin system a complexity already apparent from those components of the system about which some knowledge has accumulated and undoubtedly compounded by the components about which essentially no accepted and analyzable knowledge exists It thus becomes evident that insulin deficiency need not be based upon a subnormal amount or number of pancreatic islets or even of insulin producing beta cells Deficiency could equally well result from cells normal in size but abnormal in their ability to synthesize insulin or inadequately endowed with noninsulin constituents required for insulin storage or insufficiently effective in releasing insulin from its storage form when required Lacy and Hartroft have recently emphasized the possible importance of the several membranous structures separating the beta granules from the blood stream [*104a*] Deficiency could also result from an abnormal system for insulin transport from the presence of excess insulin binding or insulin neutralizing proteins from inappropriate insulin destruction or from excessive requirements for insulin secondary to for example the abnormal secretion of other hormones such as somatotropin or glucagon Finally deficiency could follow the existence of inadequate interaction between insulin and insulin responsive tissues whether because of abnormal binding sites for insulin or because of other dysfunctions at the tissue level

As stated above under Endocrine Defects in Diabetes *some decrease in total mass of the beta cells of the islets of Langerhans* can be demonstrated with appropriate techniques in the majority of patients with diabetes mellitus [*8 65 66*] Nonetheless in some instances this decrease is not apparent and in many instances its degree is difficult to reconcile with the requirement for removal of 90 per cent of the pancreas in the surgical induction of experimental diabetes Accordingly great interest attaches to the careful studies of Wrenshall and his collaborators [*67 77 105–107*] who have quantitated the amount of insulin which can be extracted from the pancreas of diabetic patients at autopsy These authors have applied a standard extraction and assay procedure to the pancreas obtained at autopsy from 170 nondiabetic and 170 diabetic patients the age variation being extensive in both groups [*106 107*] *Some decrease in extractable insulin was found in the majority of diabetic patients when compared with the control group but not in all* Furthermore there appeared to be a rather clear differentiation of the total diabetic group into two subgroups As stated by Wrenshall these measurements clearly indicated that a gross lack of endogenous insulin is characteristic

an interaction with a small molecular basic protein This type of interaction would thus be comparable to that existing in the beta cells and *could* produce a specific carrier system in equilibrium with pancreatic insulin on the one hand and tissue receptors on the other Individuals who have been treated with insulin of animal origin for prolonged periods of time develop serum protein fractions which "bind insulin These appear to be γ globulins and thus may well represent antibodies [*91–93*] Berson has reported the interesting fact that the insulin antibody complex is dissociable at least in part There can be little doubt but that a number of proteins which occur in serum have been shown to "bind insulin and could affect over all insulin balance either by neutralizing insulin activity or by acting as insulin "carriers

In addition to the insulin protein interactions just described factors which affect the biologic activity of insulin without demonstrably altering its physical properties have also been reported [*94 95*] The presence of some of these factors the nature of which is as yet but poorly understood has been related to diabetic ketoacidosis [*96*] or to the functional activity of the anterior pituitary or adrenal [*97 98*] glands One of these factors appears to be lipoprotein in nature [*97*]

*Inactivation*

A metabolic regulator is unlikely to be effective unless it is not only rapidly made available but also rapidly destroyed Many tissues among which the liver and the renal tubules are outstanding are capable of inactivating insulin Proteolytic enzymes which exhibit at least some specificity toward insulin have been described and extensive investigations of these systems as well as of factors favoring or antagonizing insulin destruction have been carried out [*99–102*] It is interesting that insulin ' antibodies of the type described above although presumably interfering with the effectiveness of insulin by preventing its interaction with insulin responsive tissues simultaneously decrease the rate of insulin destruction [*91, 92*]

*Interaction with Responsive Tissues*

Although present concepts of the action of insulin will be extensively discussed in the next section it should be stated here that a considerable body of evidence indicates that at least under certain conditions insulin binding precedes or accompanies insulin effectiveness in responsive tissues [*103 104*] Thus it is conceivable that insulin release transport action and inactivation all are part of a system of equilibriums between insulin on the one hand and insulin receptors on the other Evidence presently accumulating in this laboratory in collaboration with H N Antoniades suggests that indeed this might be the case

been clearly outlined by Field [*114*] It should perhaps be recalled here that as yet no convincing evidence for genetic separation of these two clinical types has been presented

That nonresponsiveness of some or all insulin dependent tissues may be involved in some instances of diabetes mellitus has been recently suggested in a number of instances particularly in the rare syndrome of *associated lipoatrophy and diabetes* first described by Lawrence [*115–119*] Furthermore the recent report by Miller [*117*] of the occurrence of lipoatrophic diabetes in two siblings suggests the possibility that this syndrome is genetically distinct

## METABOLIC EFFECTS OF INSULIN

Soon after Banting and Best [*120*] reported in 1921 the preparation of pancreatic extracts which were successful in lowering blood glucose and in prolonging the life of pancreatectomized dogs insulin became available to several laboratories in Canada the United States and Great Britain A complete bibliography [*121*] published in 1923 contained only 15 articles but from these it was already evident that insulin *primarily* caused an increased rate of extraction of glucose from blood Hepburn and Latchford [*122*] soon demonstrated an effect in vitro namely a three to fourfold increase in glucose uptake by the isolated perfused heart after insulin was added to the system It is not surprising that this preparation should have been among the first used for prior to the availability of insulin it had been reported that hearts excised from diabetic animals extracted less glucose from the perfusing medium than did normal hearts [*123*] and several years before the achievement of Banting and Best Clark [*124*] reported that the connection of a perfused pancreas into the perfusing circuit accelerated the rate of glucose disappearance!

By 1925 it had been demonstrated that insulin accelerates the rate of glucose uptake by extrahepatic tissues other than the heart as measured by arteriovenous differences across an extremity [*125*] or by an increased rate of disappearance in the hepatectomized dog [*126*] and in the eviscerated cat [*127*] It had also been shown that insulin acutely increases the respiratory quotient and Kellaway and Hughes [*128*] suggested that much of the disappearing glucose was converted to an oxygen poor substance such as fat

Since the pioneering studies mentioned above the bibliography of insulin has become diversified and extraordinarily extensive yet it would still seem that insulin primarily effects an accelerated glucose uptake by certain tissues With the refinement of physiologic techniques and especially since the advent and now widespread use of radioactive isotopes numerous metabolic processes have been reported to be altered following the addition of insulin to experimental systems whether to

of human beings who acquire diabetes during the period of normal growth (approximately the first twenty years of life) Diabetics diagnosed thereafter differed in that a considerable amount of insulin was present in the pancreas at autopsy in a majority of such persons [106] Mean values presented in 1952 are shown in Fig 3-4

Pancreatic insulin extractable at autopsy, need not of course reflect the secretory ability of the pancreatic islets Indeed this measurement is more likely to relate to the adequacy of insulin storage It is of interest therefore to find that several investigators have reported the presence of subnormal quantities of circulating insulin like activity in serum or plasma of patients whose diabetes developed during the period of normal growth while circulating insulin like activity was near normal or greater than normal in patients with adult onset of the disorder [108 109] Other observers have failed to find uniformly decreased plasma insulin like activity in juvenile diabetic patients [110 111]

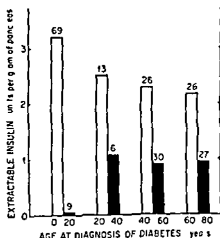



Fig 3-4 Variation in the average extractable insulin of human pancreas at autopsy in diabetes (black strips) with age at diagnosis of diabetes Values for normal nondiabetic controls at death are shown as white strips The number of subjects in each group is shown above the strip (*From Wrenshall [67]*)

On the basis of the studies just summarized as well as others [112 113] *there is a widespread tendency to differentiate two major clinical types of diabetes in man* The first type declares itself most frequently during the period of growth and is typically associated with weight loss severe ketoacidosis on insulin withdrawal normal sensitivity to insulin or even hypersensitivity nonresponsiveness to hypoglycemic sulfonylureas and evidence of decreased pancreatic and plasma insulin content The second type declares itself preferentially during adult life and is typically associated with obesity absence of severe ketoacidosis on insulin withdrawal subnormal sensitivity to insulin responsiveness to hypoglycemic sulfonylureas and poor if any evidence of decreased pancreatic and plasma insulin content Intermediate forms exist and indeed are frequent It is entirely conceivable that these two clinical types of diabetes differ primarily from a quantitative point of view and represent complete (growth-onset ketotic) or incomplete (adult nonketotic) insulin deficiency However it is equally conceivable that the adult nonketotic type of diabetes in man represents relative insulin deficiency *secondary* to the existence of other etiologic factors resulting in an increased requirement for insulin The present status of this important controversy has recently

Embden [132] and Meyerhof [133] 20 years later defined the part played by phosphorylated intermediates in the process of fermentation Since then it has become apparent that mammalian organisms likewise require the formation of phosphate esters prior to the stepwise metabolism of carbohydrates

Before glucose can be metabolized it has to enter the cell Once inside the cell it is phosphorylated The enzyme which catalyzes the phosphorylation of glucose is hexokinase and the reaction as carried out in tissue extracts or purified enzyme systems requires high-energy phosphate (an acid anhydride) to form glucose 6-phosphate (Fig 3 5) It also is favored by the presence of magnesium and potassium ions The enzyme has been found in all tissues examined In liver it is frequently termed less generally 'glucokinase since this tissue also contains

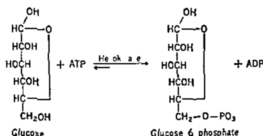



Fig 3-5 Activation of glucose to glucose 6-phosphate

pecific kinases for galactose and fructose The reaction is exergonic and thus predominantly unidirectional (at physiologic concentrations of all reactants) toward the formation of glucose 6 phosphate

*Metabolism of Glucose 6 Phosphate*

Glucose 6-phosphate has at least five different metabolic pathways open to it Some of these pathways are common to all tissues while some tissues lack the enzymes required for one or more of them Each tissue characteristically appears to exhibit its own unique pattern which may vary depending upon the organism s hormonal or nutritional state at any given time Schematically these pathways are shown in Fig 3 6

*Glucose 6 Phosphatase*

The first and simplest route open to glucose 6-phosphate metabolism is that catalyzed by the single enzyme glucose 6-phosphatase resulting in the cleavage of glucose 6-phosphate into free glucose and inorganic phosphate This reaction is the principal source of nonalimentary glucose in the mammalian organism and provides circulating glucose during periods of carbohydrate deprivation The enzyme is present primarily in liver although its concentration is similar in the kidney but because

the total animal an eviscerated or hepatectomized preparation a perfused organ or limb (either isolated or *in situ*) an isolated tissue preparation in vitro or even in some instances separated cells subcellular particles or enzymes The number and diversity of the reported effects suggest that insulin controls a single widespread and fundamental reaction which in turn affects numerous subsidiary reactions or that it exerts direct effects upon multiple physiologic functions To date experimental evidence favors the former concept namely that insulin primarily affects a single process carbohydrate entry into cells

*Primary and Secondary Effects*

Current developments in biochemistry and endocrinology have tended to support the thesis that hormones exert their primary action upon a single enzymatic reaction or reaction type and that subsequent effects are secondarily linked to this primary event An excellent specific example is that of the increased hepatic phosphorylase activity demonstrated after epinephrine or glucagon administration [129] In this instance the primary effect of epinephrine and glucagon is that of inducing the formation of 3 5 -cyclic adenine nucleotide a substance which favors the accumulation of the active form of the activating enzyme (dephosphophosphorylase kinase) which in turn catalyzes the phosphorylation of inactive phosphorylase (dephosphophosphorylase) to active phosphorylase [130] The increased phosphorylase then accelerates the catalysis of glycogen breakdown and in the presence of glucose 6-phosphatase raises blood glucose In this instance therefore it is possible to trace in some detail the links which connect the observed physiologic effect of epinephrine and glucagon i e hyperglycemia with the primary or molecular site of action of these hormones

It is tempting to speculate that insulin similarly is primarily concerned with a single important and obviously vital reaction and that the numerous observed metabolic effects are merely reflections of this reaction

In order to discuss primary and secondary metabolic alterations produced by insulin (or by insulin lack) some pathways of carbohydrate fat and protein metabolism and the intimate interrelations between these pathways must first be sketched The following brief section will stress the interdependence of the pathways discussed rather than the individual enzymatic reactions themselves For further discussion the reader is referred to the bibliography and to standard biochemical references

SOME PATHWAYS OF INTERMEDIARY METABOLISM

*Phosphorylation of Glucose*

Harden and Young [131] at the turn of the century described the role of phosphate in the utilization of sugar by living organisms and

leading to glycogen breakdown [139] Since enzymes only accelerate the attainment of a given equilibrium this observation similarly suggested that glycogen synthesis must occur by a different route while phosphorylase under physiologic conditions serves to catalyze glycogen breakdown only [140]

Beloff Chain [141] interpreted a series of experiments in muscle and liver as suggesting that glycogen synthesis occurs by direct polymerization of glucose to disaccharides trisaccharides and progressively larger molecules rather than via phosphorylated intermediates In 1957 Leloir and Cardini [142] demonstrated glycogen synthesis in liver preparations in the presence of uridine diphosphoglucose as cofactor and demonstrated in a subsequent publication that this pathway could account for the rate of glycogen synthesis prevailing in liver in vivo The uridine diphosphoglucose (UDPG) pathway has also been demonstrated in muscle and its equilibrium has been reported to favor glycogen synthesis [138] It is as yet too early to evaluate the detailed importance of the UDPG-catalyzed mechanism in the intact animal or in tissues other than liver and muscle The pathways of glycogen synthesis and breakdown which have been discussed are outlined in Fig 3 7

Once glycogen has been formed the 1 4 glycosidic links may be altered by branching enzyme (amylo-1 4 1 6 transglycosidase) to form 1 6 links thereby originating new chains of 1 4 links Thus glycogen consists of a central reducing aldehyde from which spring chains of 1 4 links and less frequent 1 6 links or branch sites The ratio of 1 4 links to 1 6 links is approximately 8 1 but this figure is an average and there is probably considerable inhomogeneity [143] As expected the outer tiers of the glycogen molecule are metabolically more active as has been shown by the isotopic carbon studies of Stetten and Stetten [144]

In the breakdown (or phosphorolysis) of glycogen by phosphorylase successive 1 4 links are cleaved to form glucose 1 phosphate until a 1 6 link is encountered To rupture this bond another enzyme amylo 1 6 glucosidase is required This results in the formation not of glucose 1 phosphate but of free glucose This free glucose is presumably rephosphorylated in peripheral tissues in liver it may be a source of blood glucose though obviously a much less important one than that resulting from glucose 6 phosphatase activity Much additional information concerning glycogen metabolism is presented in Chap 6 Glycogen Deposition Diseases

*Glycolysis*

The third route of glucose 6-phosphate metabolism is the now classical glycolytic route (Fig 3 8) also termed the *Embden Meyerhof* or *Embden Meyerhof Parnas Cori* pathway after the investigators who contributed to its elucidation Glucose 6-phosphate is isomerized to

of its smaller size the kidney contributes no more than one tenth of the production of blood glucose The enzyme has also been described in intestinal mucosa and placenta and to a much lesser extent in other tissues [134] Its probable presence in the beta cells of the islets of Langerhans has already been discussed Curiously the enzyme is not found in the soluble fraction as are all the other enzymes directly involved in the metabolism of glucose 6-phosphate but is associated with the microsomes [135] The physiologic role of this enzyme in controlling the level of blood glucose will be discussed below, in the section devoted to liver metabolism

*Synthesis of Glycogen*

A second pathway open to glucose 6 phosphate is glycogen synthesis Glucose 6-phosphate first forms glucose 1 phosphate, in the presence of

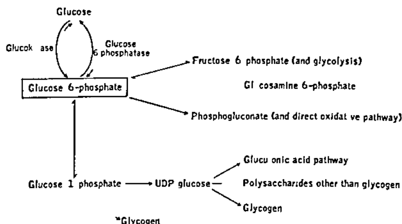



Fig 3 6 Metabolic pathway of glucose 6-phosphate

phosphoglucomutase a reaction in which glucose 1 6 diphosphate is a necessary cofactor In the presence of active phosphorylase glucose 1 phosphate condenses with the nonreducing end of a glycogen chain forming a new 1 4 link with loss of inorganic phosphate Until recently it was generally considered that this reaction governed both glycogen synthesis and mobilization the direction of the net reaction depending primarily on the relative concentrations of glucose 1 phosphate and inorganic phosphate [136] When the intracellular concentrations of these two substances were actually measured it was found that their relative concentrations were such as always to favor glycogenolysis [137 138] Furthermore it was well known that epinephrine induced or glucagon induced increases in phosphorylase activity always increased glycogenolysis never the synthesis of glycogen This indicated that phosphorylase activity is rate limiting only in the sequence of events

fructose 6 phosphate which is further phosphorylated to form fructose 1 6 diphosphate in the presence of 6-phosphofructokinase This phosphorylation similar to the initial phosphorylation of glucose requires the donation of high-energy phosphate from ATP to form a low-energy ester The reaction is essentially unidirectional The reverse reaction the conversion of fructose 1 6-diphosphate to fructose 6-phosphate and inorganic phosphate is catalyzed by another enzyme fructose 1 6-diphosphatase also an essentially unidirectional reaction The participation of two enzymes in the control of the interconversion of fructose 6 phosphate and fructose 1 6 diphosphate is of considerable importance

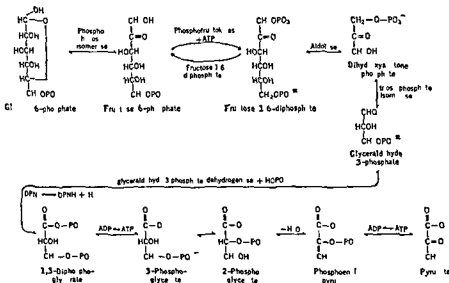



Fig 3 8 Pathway of glycolysis

since it provides a means of controlling the over all rate *and* direction of the glycolytic pathway Tissues which are mainly glycolytic such as muscle or brain, contain the highest concentration of phosphofructokinase [145] Conversely high levels of fructose 1 6-diphosphatase would be expected in whatever tissue exhibits the greate t degree of gluconeogenesis from three-carbon precursors The be t source of this enzyme is indeed the liver [146]

Fructose 1 6-diphosphate is then plit in the presence of aldolase into two phosphorylated three-carbon intermediates the upper three carbons forming dihydroxyacetone pho phate and the lower three glyceraldehyde 3 pho phate The e two compounds are interconvertible in the presence of the enzyme triose pho phate isomerase Glyceraldehyde 3-pho phate is then dehydrogenated in the presence of pho pho glyceraldehyde de-

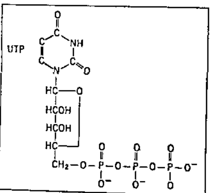



Fig 3 7 Pathway of glycogen synthesis and breakdown

upper two carbons of the xylulose 5 phosphate are cleaved in the presence of transketolase and are condensed onto ribose 5 phosphate to form a even carbon ketose sedoheptulose 7 phosphate The remaining or bottom three carbons of the xylulose 5 phosphate form glyceraldehyde

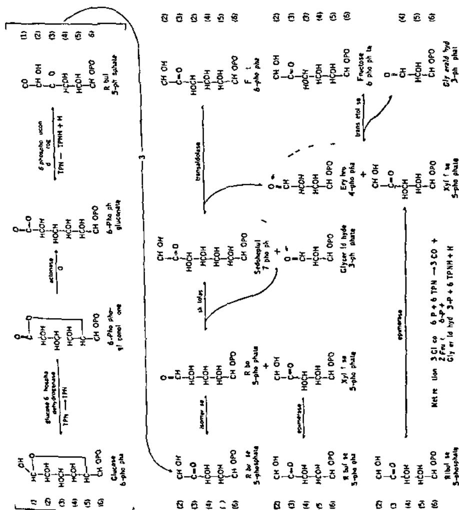


Fig 3 9 The phosphogluconate-oxidative (direct oxidative) pathway

3-phosphate The sedoheptulose 7 phosphate is then cleaved in the presence of transaldolase the upper three carbons condense with glyceraldehyde 3 phosphate to form fructose 6-phosphate which is either isomerized to glucose 6 phosphate or further metabolized by the glycolytic pathway The remaining four-carbon sugar the lower part of the original sedoheptulose 7 phosphate erythrose 4 phosphate accepts a two-carbon fragment from a third pentose phosphate to form fructose 6-phosphate

hydrogenase and diphosphopyridine nucleotide (DPN) as hydrogen acceptor with the simultaneous introduction of a second phosphate into the molecule to form 1 3 diphosphoglycerate This compound subsequently loses one phosphate residue in the presence of ADP to form 3 phosphoglycerate and ATP In the succeeding steps the remaining phosphate is transferred to the 2 position and 2 phosphoglycerate is transformed into phosphoenolpyruvate with the loss of 1 molecule of water In the final step phosphoenolpyruvate with ADP, is converted to pyruvate and ATP

In summary the conversion of 1 mole of glucose to 2 moles of pyruvate requires 1 mole of ATP for each of the phosphorylations, while the metabolism of each of the two trioses to pyruvate yields 2 moles of ATP and 1 mole of reduced DPN In the absence of oxygen the hydrogens of reduced DPN can be conveniently disposed of by transferring them to pyruvate in the presence of the enzyme lactic acid dehydrogenase resulting in the formation of lactate and in the regeneration of oxidized DPN The over all reaction under these conditions may be written




For each mole of glucose metabolized by the glycolytic pathway there is a net production of 2 moles of ATP This energy accounts for a small fraction only of the total energy available from glucose but it is available in the absence of oxygen Therefore the term most commonly used to refer to this sequence of reactions is *anaerobic glycolysis* since it can indeed proceed "anaerobically

*Phosphogluconate oxidative Pathway*

Names used for this route of glucose 6-phosphate metabolism include in addition to *phosphogluconate oxidative pathway* the terms *direct oxidative pathway phosphogluconate pathway Lipmann Dickens pathway pentose phosphate pathway* The colloquial term frequently applied to this metabolic route is that of the shunt

Glucose 6 phosphate first undergoes dehydrogenation to form 6-phosphogluconolactone a cyclic ester which in turn is enzymatically hydrolyzed to 6 phosphogluconate Next 6 phosphogluconate is dehydrogenated and decarboxylated producing ribulose 5 phosphate These two initial reactions of the pathway result in the removal of the aldehyde carbon of glucose as $CO_2$ and in the further removal of four hydrogens The specific acceptor for these hydrogens is triphosphopyridine nucleotide (TPN) resulting in the formation of two molecules of reduced TPN The importance of this by product of the oxidative steps of this pathway will be shown during the discussion of the synthesis of fatty acids

The further metabolism of ribulose 5 phosphate as shown in Fig 3 9 requires the rearrangement of two molecules one isomerized into ribose 5 phosphate and the other epimerized into xylulose 5 phosphate The

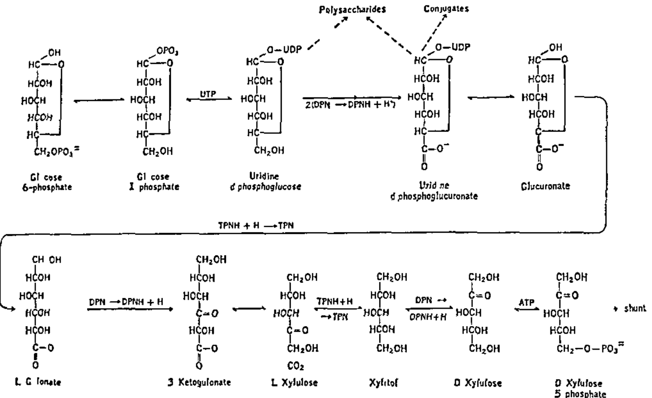
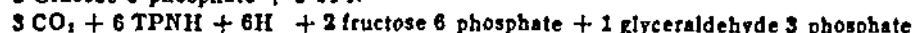



Fig 3 10 Glucuronic acid pathway

and glyceraldehyde 3 phosphate The over all reaction may be written


3 Glucose 6 phosphate + 6 TPN ⟶


$3\ CO_2 + 6\ TPNH + 6H^+ + 2$ fructose 6 phosphate + 1 glyceraldehyde 3 phosphate

The initial oxidative steps are essentially irreversible hence the metabolism of glucose 6-phosphate by this pathway proceeds only in the direction of pentose phosphate formation However, all the other steps are reversible For example by the transaldolase reaction fructose 6-phosphate can transfer its upper three carbons to erythrose 4 phosphate to form sedoheptulose 7 phosphate or by the transketolase reaction it can transfer its upper two carbons to glyceraldehyde 3 phosphate to form xylulose 5-phosphate and erythrose 4 phosphate In tissues such as muscle which lack significant amounts of the two enzymes needed to dehydrogenate glucose 6-phosphate ribose synthesis occurs by transketolation and transaldolation from fructose 6-phosphate

*Glucuronic Acid Pathway*

At the time of this writing it is difficult to assess the quantitative importance of the glucuronic acid pathway in the total organism or in individual tissues The existence of this pathway has been established beyond reasonable doubt by Horecker and Hiatt [147] It first requires the conversion of glucose 6-phosphate to glucose 1 phosphate which subsequently reacts with uridine triphosphate (UTP) to form uridine diphosphoglucose (UDPG) and pyrophosphate Uridine diphosphoglucose may undergo several reactions including the formation of glycogen epimerization at the fourth carbon of glucose to form uridine diphosphogalactose possibly epimerization and metabolism in other positions to form sugars such as idose mannose or galactosamine and in the glucuronic acid pathway oxidation at the sixth carbon of glucose with two molecules of DPN as coenzyme to form uridine diphosphoglucuronic acid (UPDGA) and two molecules of DPNH This UDPGA may then be used in the synthesis of polysaccharides and mucopolysaccharides or it may serve as the glucuronic acid donor in conjugating reactions with substances such as bilirubin phenols or steroids or it may be further metabolized as shown in Fig 3 10 In this sequence of events uridine diphosphate is split off and free glucuronic acid is reduced in the first position to form L-gulonic acid This in turn is oxidized to 3 ketogulonic acid or 2 ketogulonic acid then decarboxylated to L-xylulose In animals such as the rat 3 ketogulonic acid or its isomer 2 ketogulonic acid appears to be the precursor of ascorbic acid L-Xylulose is reduced to xylitol a symmetric molecule then reoxidized to form D-xylulose After phosphorylation in the presence of D xylulose kinase and ATP xylulose 5 phosphate can be metabolized by the reactions described in Fig 3 9 i e the reactions of the phosphogluconate-oxidative pathway

lenged since it has been shown that the presence of $CO_2$ is a necessary requirement the intermediate product formed being malonyl CoA [148]. Malonyl CoA then condenses with acetyl CoA (or more generally with an acyl CoA) to form the corresponding branched chain $\alpha$ carboxyl $\beta$ keto fatty acid with subsequent reduction of the keto group dehydration and reduction of the double bond and finally cleavage to $CO_2$ of the *same* carboxyl originally derived from $CO_2$. The over all reaction

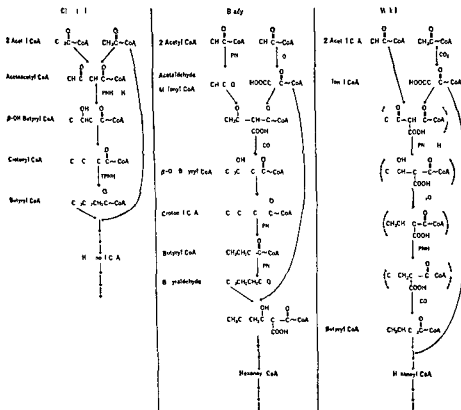



Fig 3-11 Pathways of fatty acid synthesis (early 1960)

thus also results in elongation of the acyl residue by two carbons without evidence of the $CO_2$ carbon in the end product! In the isolated system *TPNH again serves as coenzyme in the reductive steps* Although clarification of the quantitative importance of this pathway is still required it is of interest to consider that if fatty acids were synthesized primarily by the malonyl pathway and primarily catabolized by reversal of the classical pathway synthesis and breakdown could be more readily controlled the over all pattern being analogous to synthesis of glycogen

*Metabolism of Pyruvate*

Under aerobic conditions the pathways of glucose dissimilation converge to form pyruvate Pyruvate is oxidatively decarboxylated to form acetyl coenzyme A (acetyl CoA) and DPNH This essentially irreversible and complex step requires the presence of a conjugate of thiamine and lipoic acid and of several fractions with enzymatic activity Acetyl CoA has several pathways open to it of which four are of major quantitative importance oxidation in the tricarboxylic acid cycle incorporation into long chain fatty acids incorporation into cholesterol and metabolism to acetoacetate and $\beta$ hydroxybutyrate

*Tricarboxylic Acid Cycle*

Acetyl CoA condenses with oxaloacetate to form citric acid which is then metabolized further in the presence of the enzymes which comprise the tricarboxylic acid (TCA) cycle also called the Krebs or the citric acid cycle Each turn of this cycle leads to the incorporation of one molecule of acetate while two molecules of $CO_2$ are given off and while two molecules of DPNH one molecule of TPNH and one high energy phosphate bond are generated and two further hydrogen atoms are directly incorporated into the respiratory enzyme chain More than 90 per cent of the energy of the glucose molecule is derived by the further metabolism of pyruvate by the TCA cycle

*Synthesis of Fatty Acids*

Acetyl CoA may condense with a second molecule of acetyl CoA cleaving off free coenzyme A with formation of acetoacetyl CoA In fatty acid–synthesizing tissues (Fig 3 11) and according to concepts generally accepted until very recently acetoacetyl CoA is then reduced stepwise first in the presence of DPNH to form $\beta$ hydroxybutyryl CoA which is dehydrated to crotonyl CoA Crotonyl CoA is then reduced *in the presence of TPNH* to butyryl CoA The process is then repeated two carbons at a time beginning by condensation with another molecule of acetyl CoA to form the CoA derivative of the $\beta$ keto acid with two more carbons with subsequent reduction to the $\beta$ hydroxy acid dehydration to the unsaturated acid and finally reduction to the saturated fatty acid After a predetermined chain length has resulted from these successive elongations free coenzyme A may be regenerated while esterification of the fatty acid residues with L-$\alpha$ glycerophosphate may lead to the formation of phosphatidic acids and subsequently to the formation of triglycerides or phospholipids Esterification of the fatty acid residues may also occur with other alcohols such as cholesterol

Recently this classical pattern of fatty acid synthesis has been chal

mevalonic acid once this intermediate is formed cholesterol synthesis appears to proceed almost spontaneously [150] The problem of cholesterol synthesis and metabolism is more fully discussed in Chaps 16 and 20

*Ketogenesis*

Acetoacetyl CoA and β hydroxybutyryl CoA instead of undergoing further metabolism to fatty acids or cholesterol by successive elongation of their carbon chain may also form free acetoacetate and β hydroxybutyrate Liver and kidney are equipped with a series of enzymes effecting deacylation' and it has recently been suggested by Lynen [151] that the reaction might be a complex one involving first the synthesis of β hydroxy β methyl glutaryl CoA with subsequent liberation of acetoacetate acetyl CoA reentering the acetyl CoA pool This reaction sequence resulting in the accumulation of acetoacetate becomes quantitatively important when *acetoacetyl CoA accumulates under conditions* which do not favor the synthesis of fatty acids and to a lesser extent cholesterol This is the case for instance in fasting or in the diabetic state as will be further emphasized below When acetoacetate accumulates in acetoacetate-forming tissues it diffuses into the blood stream and is carried to all tissues Before being utilized for further oxidation or for synthetic reactions it has to be reactivated either (in all tissues) by direct reaction with coenzyme A in the presence of ATP or (primarily in muscle) by transfer of coenzyme A from succinyl CoA formed in the tricarboxylic acid cycle The latter reaction is energetically more economical and whereas it does not occur in liver it predominates in muscle thereby establishing a transport system for acetoacetate from liver to muscle under appropriate conditions

*Dicarboxylic Acid Shuttle*

Another important metabolic link between glycolysis and the TCA cycle is frequently termed the *dicarboxylic acid shuttle* Although the feasibility of the direct reaction has been recently suggested [153] the direct conversion of pyruvate to phosphoenolpyruvate is difficult since the equilibrium greatly favors the formation of pyruvate because of the free-energy levels of the reactants [152] Studies with carbon 14-labeled substrates have shown that tissues which synthesize glucose (e g liver placenta) bypass this energy block by a reductive carboxylation of pyruvate to malate in the presence of TPNH Malate an intermediate of the TCA cycle is oxidized by DPN to oxalacetate which is in turn decarboxylated and phosphorylated in the presence of inosine or guanosine triphosphate to form phosphoenolpyruvate Thus the direct conversion of pyruvate to phosphoenolpyruvate is bypassed and in the process TPNH is oxidized to TPN and DPN reduced to DPNH as shown in Fig 3-13

by the uridine diphosphoglucose pathway and breakdown by the phosphorylase mechanism

*Synthesis of Cholesterol*

Acetyl CoA is also used for the synthesis of cholesterol Through a series of reactions, as yet incompletely understood [149] acetyl CoA condenses with acetoacetyl CoA to form β-hydroxy β methyl glutaryl CoA which is reduced by TPNH to mevalonic acid which is in turn

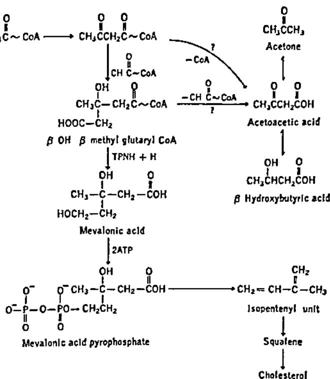



Fig 3-12 Pathways of cholesterol synthesis and ketogenesis

decarboxylated to form an ' isoprenoid i e an isopentenyl derivative similar in structure to isoprene as shown in Fig 3 12 Three of these isoprenoid units condense to form a 15-carbon intermediate of which two condense end to end to form squalene which folds condenses splits off three methyl groups and after a single reduction in the side chain results in the formation of cholesterol [149] Cholesterol may then undergo many further reactions which include the synthesis of steroid hormones bile acids or vitamin D or it may remain unchanged as cholesterol free or esterified Recent studies have suggested that the rate limiting step in this sequence of reactions is the formation of

to fatty acids according to the ' classical pattern of fatty acid synthesis requires both DPNH and TPNH while the ' malonyl ' pathway probably requires primarily TPNH There are apparently ample sites for DPNH synthesis but only two major sites of TPNH synthesis One is the oxidative decarboxylation of glucose 6 phosphate in the first two reactions of the shunt The other is the isocitric dehydrogenase reaction in the TCA cycle and since this process is associated with mitochondrial enzymes this TPNH may not be so available for reductive synthetic mechanisms as is that generated by the shunt It should also be pointed

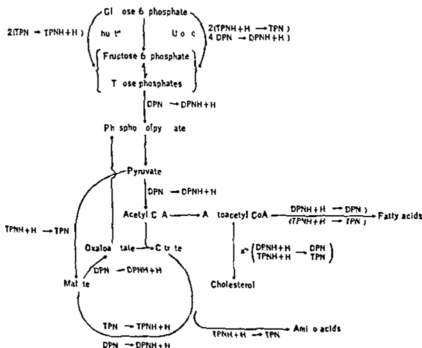


Fig 5-14 Some aspects of hydrogen balance

out that there are at least two pathways which may serve to convert TPNH to DPNH One is the uronic acid pathway which utilizes 2 moles of TPNH and produces 4 moles of DPNH for each hexose phosphate metabolized to pentose phosphate The other pathway is the fixation of $CO_2$ by pyruvate in the dicarboxylic acid shuttle to form malate This latter process requires TPNH DPNH being generated by oxidation of the malate to oxalacetate



Since TPNH is required for fat synthesis a tissue which utilizes hydrogen in this form e g a tissue with considerable glucuronic acid pathway activity would not be expected to carry on active lipogenesis

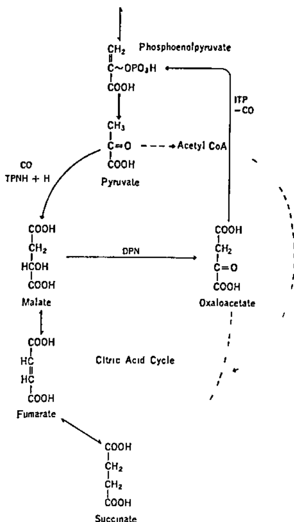
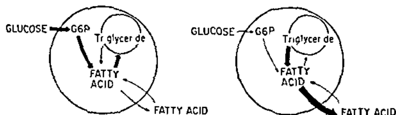


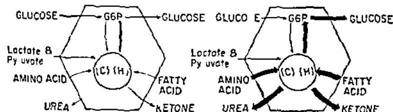

Fig. 3-13 Dicarboxylic acid shuttl

*Hydrogen Balance*

Although it is customary to think of intermediary metabolism in terms of the carbon atoms involved or in terms of oxygen used it is becoming apparent that one of the major means used for the regulation of intermediary metabolism is the control of hydrogen flow or balance between substrates [164] intermediates and water with the help of some highly specific coenzymes among which DPN and TPN are of particular importance to this discussion A summary of the roles of DPN and TPN in the reactions which have been outlined is shown in Fig 3 14 It should again be stressed that the conversion of acetoacetate

must be assumed that gluco e phosphorylation and dephosphorylation are occurring at identical rates under these conditions Below this level of glycemia liver produces glucose, and above this level glucose is taken up and deposited as glycogen Glucose 6 phosphatase activity is increased



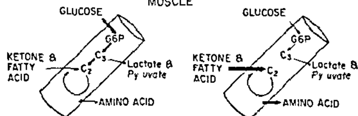




Fig 3-15 *Effect of the diabetic state on some path ays of intercellulary m tabolism in liver muscle an l a l pose tis ue*

by fa ting and is al o elevated in diabete The effective activity of its oppo ing partner glucokinase is mea ured by the pho phorylation mechanism i decreased by fa ting or in diabetes As a re ult in the diabetic tate liver may continue t produce gluc se even in the presence of severe hyperglycemia wherea the normal liver removes glucose and depo its it as glycogen under these c nditions [I57]

Likewise a tissue actively fixing $CO_2$ as in hepatic gluconeogenesis from pyruvate would be expected to exhibit relatively poor lipogenesis and indeed lipogenesis in liver appears to be roughly inversely proportional to the activity of simultaneously occurring gluconeogenesis!

The enzymatic interconversion of DPNH and TPNH has been described in several tissues. The enzyme involved transhydrogenase does not appear to be sufficiently active to equilibrate effectively intracellular DPNH and TPNH. Recent evidence suggests that the activity of this interconversion may play a major role in the hormonal regulation of metabolic pathways. Thus placental transhydrogenase is activated by estrogens [155] and there is evidence that other steroids may likewise exert their metabolic effects by acting as cofactors for this enzyme in specific tissues.

### METABOLISM IN INDIVIDUAL TISSUES

The previous paragraphs have briefly outlined some aspects of over all metabolic pathways their direct interrelations and the indirect integration achieved by the coenzymes DPNH and TPNH. In the following section three typical tissues will be discussed liver muscle and adipose tissue. These are selected from a metabolic viewpoint as quantitatively the most important tissues for over all energy metabolism and also because they exemplify three different patterns of metabolism. Furthermore they are the tissues most actively studied in diabetes. Liver is primarily gluconeogenic muscle is primarily glycolytic (its main function is the derivation and storage of chemical energy which can be immediately or eventually converted to mechanical energy) and adipose tissue controls the synthesis and storage of fatty acids as triglyceride and the mobilization of these fatty acids for release into the blood stream. Some of these relationships both in the normal and in the diabetic state have been summarized in Fig. 3 15

*Liver*

The role of the liver in carbohydrate metabolism as originally and correctly defined by Claude Bernard over 100 years ago is twofold. It is able to store carbohydrate as glycogen and to release this carbohydrate into the circulation when needed; it is also able to convert noncarbohydrates such as proteins amino acids or intermediates such as glycerol or lactate into glucose and glycogen. The enzymatic machinery of the liver is therefore geared to these activities. As stated previously glucose 6-phosphatase is present in liver and catalyzes the final step resulting in the production of blood glucose.

Soskin and his collaborators [156] have demonstrated that glucose uptake or production by liver from normal fed animals ceases at the blood glucose concentration of approximately 150 mg per 100 ml. Thus it

lates as acetoacetyl CoA and ultimately as acetoacetate From this one might tentatively conclude that *ceteris paribus* the generation of one acetoacetate molecule from fatty acid catabolism could account for the synthesis of two glucose molecules from pyruvate

*Muscle*

With regard to the aspects of intermediary metabolism considered here muscle metabolism is relatively simpler than liver metabolism Muscle appears to lack the enzymes required for the oxidative steps of the shunt although it does exhibit other enzymatic mechanisms of this pathway and probably synthesizes pentoses by means of the transaldolase and transketolase reactions [*161*] In muscle there is essentially no fatty acid synthesis (a finding consonant with the absence of the TPNH generating oxidative steps of the shunt) and studies with isotopic tracers have revealed the probable absence of the dicarboxylic acid shuttle [*162*] What little pyruvate is incorporated into muscle glycogen is apparently incorporated by direct reversal of the equilibrium between phosphoenol pyruvate and pyruvate Finally muscle lacks glucose 6-phosphatase and is therefore a purely glucose utilizing never a glucose-producing tissue Besides glucose muscle readily metabolizes ketone bodies and fatty acids and in vitro studies have shown that in certain metabolic states such as diabetic acidosis all the metabolic energy of muscle may be derived from the metabolism of these nonglucose fuels [*163*]

*Adipose Tissue*

Adipose tissue represents another pattern of metabolism one centered around fatty acid synthesis and release Experiments with isotopically labeled carbon have shown that the major part of mammalian fat deposits is synthesized directly in adipose tissue from glucose and other precursors [*164*] Glucose is a particularly effective fatty acid precursor in this tissue half the glucose being metabolized by way of the shunt in order to generate the large amounts of required TPNH [*165*] Triglycerides in the form of chylomicrons are probably to a large extent incorporated directly into adipose tissue lipid [*166*] although the ester bonds may well be hydrolyzed and resynthesized in the process The second important metabolic function of adipose tissue concerns the release of fatty acids from the tissue into the circulation primarily in the form of albumin bound nonesterified (NEFA) or unesterified (UFA) or free fatty acids Much research is currently in progress concerning the mechanism of this release In general it appears that *adipose tissue releases fatty acids* especially during episodes of carbohydrate deprivation [*167*] The carbohydrate deprivation may be absolute as in the fasting state or it may be relative as in the diabetic state with hyperglycemia but

In regard to the other step in glycolysis which is controlled by two opposing enzymatic reactions it has already been stated that phosphofructokinase activity in liver is lower than in any other tissue in which it was assayed [145] Conversely, fructose 1 6-diphosphatase is readily demonstrated in liver and its activity increases in fasting in diabetes or following glucocorticoid administration [146] Likewise the activity of the dicarboxylic acid shuttle is great in liver and is increased in fasting and in diabetes in order to permit accelerated resynthesis of phosphoenol pyruvate from pyruvate In summary therefore within the sequence of reactions which comprises the glycolytic pathway each step for which two separate enzymes or enzyme systems are provided favors gluconeogenesis in hepatic tissue and this tendency is further enhanced by fasting or in the diabetic state

With respect to the direct oxidative pathway it has been repeatedly shown in studies with glucose specifically labeled with $C^{14}$ that twice as much carbon 1 as carbon 6 is recovered in $CO_2$ This suggests that one-half the glucose is metabolized by the shunt However subsequent studies in which glucose carbon was recovered in fatty acids and in glycolytic intermediates in addition to $CO_2$ suggest that the amount of glucose 6-phosphate so metabolized is more likely of the order of magnitude of 5 to 10 per cent [158] In the liver of the diabetic animal this percentage increases somewhat but the absolute amount of glucose 6-phosphate metabolized is well below normal [159]

Whether fatty acid synthesis proceeds primarily according to classical concepts (Fig 3-11) or by the malonyl CoA pathway TPNH is a required hydrogen donor at one or more of the reductive steps involved Indeed as particularly emphasized by Siperstein [160] the availability of TPNH may well play a necessary and possibly rate-limiting role in hepatic lipogenesis It is therefore not surprising to find that since only one-tenth the normal amount of glucose is metabolized by the TPNH generating pathway in diabetes lipogenesis in the liver of the diabetic animal is markedly reduced Also since gluconeogenesis from pyruvate primarily occurs by way of the dicarboxylic acid shuttle i e reactions resulting in the utilization of TPNH accelerated gluconeogenesis from amino acids lactate or pyruvate is likely to be associated with less fatty acid synthesis as indeed appears to be the case

It should be pointed out that the role of fatty acid synthesis in liver is probably relatively minor and less important than fatty acid oxidation which provides in this tissue for the necessary reductive environment required for the synthesis of glucose To synthesize one molecule of glucose from two molecules of pyruvate requires four hydrogen atoms The oxidative generation of one molecule of acetyl CoA from a long-chain fatty acid results in the availability of four hydrogen atoms in a metabolically appropriate form If total oxidation is limited acetyl CoA accumu

TABLE 3.2 RESPONSIVENESS OF INDIVIDUAL TISSUES TO INSULIN

| Tissue | Responsiveness to insulin | Major insulin effect | Effect or lack of effect demonstrated: In vivo | In vitro | In man | Concentration of insulin required, microunits/ml | Time required for initiation of effect |
|---|---|---|---|---|---|---|---|
| Muscle | | | | | | | |
| Skeletal | Present | Increased glucose uptake, increased glycogenesis. Also increased uptake of amino acid, increased incorporation of amino acids into protein | Yes | Yes | Yes | 10–1 000 | Min |
| Heart | Present | | Yes | Yes | Yes | [illegible] | |
| Adipose tissue | Present | Increased glucose uptake and oxidation, increased lipogenesis in the presence of glucose. Also decreased release of fatty acids | Yes | Yes | Yes | 10 | Min |
| Mammary gland (lactating) | Present (rat); Absent (sheep) | Increased lipogenesis in the presence of glucose | | Yes | | [illegible] 000 | Min |
| Leukocytes | Present | Increased glucose uptake | | Yes | Yes | 10 000 | 1 hr |
| Lens | Present | Increased glucose penetration | | Yes | | 10 000 | 1 hr |
| Blood capillaries | Present | Increased glucose transport | Yes | | | | 1 hr |
| Tumors | Probably present in some | Increased glucose utilization | | Yes | | 250 | 1 hr |
| Brain | Absent | | Yes | Yes | Yes | | |
| Kidney | Probably absent | | Yes | | Yes | | |
| Intestinal mucosa | Probably absent | | Yes | | Yes | | |
| Erythrocytes | Absent | | | Yes | Yes | | |
| Liver | Probably present but physiologic significance disputed | Increased glycogenesis (rat liver); increased lipogenesis; increased protein synthesis | Yes | Yes | | 10 000 | Hr |

This table represents the authors' interpretation of the available data. When the question as to demonstration of an effect is answered by "yes" rather than yes, the evidence on which the answer is based is largely indirect in nature.

inadequate utilization of glucose because of insulin lack Fatty acid release appears to be exquisitely sensitive to hormonal control, particularly to epinephrine [168] Adrenocorticotropic hormone and growth hormone have also been implicated in the activation of this release mechanism

## MECHANISM OF INSULIN ACTION

As yet the diabetic state and insulin have been mentioned only in order to place some emphasis on certain metabolic pathways and tissues The mechanism of insulin action has been for many years and still is under intense scrutiny in many laboratories Most investigators agree that in insulin responsive tissues insulin primarily facilitates the conversion of extracellular glucose to intracellular glucose 6-phosphate Somewhat over ten years ago Colowick Cori and Slein [169] postulated that insulin directly alters hexokinase activity in the presence of inhibitory pituitary or adrenal components However Levine and his collaborators [170] measuring the distribution of nonutilizable glucose analogues in eviscerated animals and Ross [171] measuring the penetration of carbohydrates into the anterior chamber of the rabbit eye as well as into the rabbit lens obtained conclusive evidence that the effect of insulin is directly on the cell ' wall and results in facilitation of the entry of glucose and substances of similar structure across this wall and into the cell The report of equal levels of hexokinase in normal and diabetic muscle [172] supports this latter mechanism of action Recent experiments by Morgan et al on the perfused rat heart [173] and by Kipnis and Cori on the isolated uncut rat diaphragm [174] suggest a dual effect of insulin resulting both in increased penetration of glucose (as measured by the accumulation of free glucose or glucose analogues inside the cell) and in the correction of a decreased intracellular phosphorylating mechanism in the diabetic state It would al o appear from these studies that the effect on permeability is immediate while the effect on phosphorylation requires time and as originally suggested by Cori is sensitive to pituitary and adrenal factors This double role of insulin is difficult to understand unless phosphorylating and penetrating (or transporting) mechanisms have one or several components in common [175] as may indeed be true Liver has no demonstrable permeability barrier to glucose [176] yet the diabetic state in liver is characterized by inability to phosphorylate glucose again suggesting some direct effect of insulin on the phosphorylating mechanism itself The reader is referred to review references for further details and discussions particularly to the recent article by Park and his collaborators [175] It should however be evident from the preceeding paragraph that for the present no categoric statement can or should be issued as to whether the effect of insulin upon glucose utilization primarily results from an action upon

TABLE 3 2 RESPONSIVENESS OF INDIVIDUAL TISSUES TO INSULIN

| Tissue | Responsiveness to insulin | Major insulin effect | Effect or lack of effect demonstrated | | | Concentration of insulin required microunits/ml | Time required for initiation of effect |
|---|---|---|---|---|---|---|---|
| | | | *In vivo* | *In vitro* | *In man* | | |
| Muscle | | | | | | | |
| Skeletal | Present | Increased glucose uptake increased glycogenesis Also increased uptake of amino acids increased incorporation of amino acids into protein | Yes | Yes | Yes | 10 1 000 | Min |
| Heart | Present | | Yes | Yes | Yes | 60 | |
| Adipose tissue | Present | Increased glucose uptake and oxidation increased lipogenesis in the presence of glucose Also decreased release of fatty acids | Yes | Yes | Yes | 10 | Min |
| Mammary gland (lactating) | Present (rat) Absent (sheep) | Increased lipogenesis in the presence of glucose | | Yes | | 2 000 | Min |
| Leukocytes | Present | Increased glucose uptake | | Yes | Yes | 10 000 | 1 hr |
| Lens | Present | Increased glucose penetration | | Yes | | 10 000 | 1 hr |
| Blood/aqueous | Present | Increased glucose transport | Yes | | | | 1 hr |
| Tumors | Probably present in some | Increased glucose utilization | | Yes | | 250 | 1 hr |
| Brain | Absent | | Yes | Yes | Yes | | |
| Kidney | Probably absent | | Yes | | Yes | | |
| Intestinal mucus | Probably absent | | Yes | | Yes | | |
| *Erythrocytes* | Absent | | | Yes | Yes | | |
| Liver | Probably present but physiologic significance disputed | Increased glycogenesis (rabbit) increased lipogenesis increased protein synthesis | Yes | Yes | | 10 000 | Hr |

This table represents the authors interpretation of the available data When the question as to demonstration of an effect is answered by yes rather than yes the evidence on which the answer is based is largely indirect in nature

inadequate utilization of glucose because of insulin lack. Fatty acid release appears to be exquisitely sensitive to hormonal control particularly to epinephrine [168]. Adrenocorticotropic hormone and growth hormone have also been implicated in the activation of this release mechanism.

MECHANISM OF INSULIN ACTION

As yet, the diabetic state and insulin have been mentioned only in order to place some emphasis on certain metabolic pathways and tissues. The mechanism of insulin action has been for many years and still is under intense scrutiny in many laboratories. Most investigators agree that in insulin responsive tissues insulin primarily facilitates the conversion of extracellular glucose to intracellular glucose 6-phosphate. Somewhat over ten years ago Colowick, Cori and Slein [169] postulated that insulin directly alters hexokinase activity in the presence of inhibitory pituitary or adrenal components. However, Levine and his collaborators [170] measuring the distribution of nonutilizable glucose analogues in eviscerated animals and Ross [171] measuring the penetration of carbohydrates into the anterior chamber of the rabbit eye as well as into the rabbit lens obtained conclusive evidence that the effect of insulin is directly on the cell "wall" and results in facilitation of the entry of glucose and substances of similar structure across this wall and into the cell. The report of equal levels of hexokinase in normal and diabetic muscle [172] supports this latter mechanism of action. Recent experiments by Morgan et al. on the perfused rat heart [173] and by Kipnis and Cori on the isolated uncut rat diaphragm [174] suggest a dual effect of insulin resulting both in increased penetration of glucose (as measured by the accumulation of free glucose or glucose analogues inside the cell) and in the correction of a decreased intracellular phosphorylating mechanism in the diabetic state. It would also appear from these studies that the effect on permeability is immediate while the effect on phosphorylation requires time and as originally suggested by Cori is sensitive to pituitary and adrenal factors. This double role of insulin is difficult to understand unless phosphorylating and penetrating (or transporting) mechanisms have one or several components in common [175] as may indeed be true. Liver has no demonstrable permeability barrier to glucose [176] yet the diabetic state in liver is characterized by inability to phosphorylate glucose again suggesting some direct effect of insulin on the phosphorylating mechanism itself. The reader is referred to review references for further details and discussions particularly to the recent article by Park and his collaborators [175]. It should however be evident from the preceeding paragraph that for the present no categoric statement can or should be issued as to whether the effect of insulin upon glucose utilization primarily results from an action upon

*capacity for glucose* As long as insulin deficiency is not complete compensation may occur with minimal to moderate hyperglycemia and thus minimal glucosuria With increasing severity of the insulin deficiency higher and higher concentrations of glucose are required to achieve compensation and the effectiveness of this increased blood glucose level is to an increasingly large extent wasted by the occurrence of rapidly mounting urinary losses Still higher levels of blood glucose can be obtained only at the metabolic cost of profuse hepatic overproduction It is evident that peripheral underutilization and hepatic overproduction of glucose are intimately related in severe states of insulin deficiency

Excessive hepatic gluconeogenesis entails the concomitant catabolism of fatty acids and protein fatty acids to provide the necessary hydrogen for reduction of pyruvate to glucose protein to contribute carbon i e the pyruvate itself either directly or indirectly Since acetate is unable to contribute a *net* amount of carbon to the reversed glycolytic sequence fatty acids are unable to contribute carbon for gluconeogenesis Since urea is the major end product of nitrogen metabolism and since acetoacetate is the major intermediary product of hepatic catabolism of fatty acids it follows from the considerations of intermediary metabolism outlined above that the synthesis of one molecule of glucose from two molecules of pyruvate should be accompanied by the accumulation of at least one molecule of acetoacetate and one molecule of urea if the pyruvate was derived from amino acids

In terms of these concepts persons with mild diabetes with minimal glucosuria (of the order of 5 to 10 gm glucose per day) should not develop ketoacidosis since the excess hepatic catabolism of fatty acids required by this glucose loss does not produce more acetoacetate than can be easily metabolized by the organism On the other hand diabetic patients who lose 100 to 200 gm glucose per day necessarily catabolize a proportional amount of fatty acids and generate large amounts of ketone bodies When the production of ketone bodies surpasses the ability of the body to utilize them they accumulate in the blood stream and since their renal threshold is minimal an increasing number is lost in the urine Since acetoacetate and β hydroxybutyrate have relatively low pK values they are predominantly excreted as anions in conjunction with cations Thus sodium and potassium are lost further aggravating the osmotic diuresis already induced by the urinary glucose loss as well as the already existing tendency to metabolic acidosis

What are the *mechanisms whereby the liver obtains the necessary* fatty and amino acids for glucose synthesis? Let us first consider relatively recent information concerning adipose tissue metabolism During carbohydrate deprivation whether due to an absolute glucose lack such as in fasting or in hypoglycemia or to a relative lack such as occurs with insulin deficiency this tissue releases nonesterified fatty acids into the

glucose transport into the cell (translocation) or from an action upon glucose phosphorylation within the cell (transformation) Past experience concerning similar controversies suggests that in time both effects will be accepted and that a connecting link between them will become apparent

Although a large number of metabolic effects of insulin in vivo and in vitro can be related directly or indirectly to a primary effect of the hormone on glucose utilization by tissues such as adipose tissue and muscle there are also some well-documented effects of insulin which cannot be readily explained on this basis alone The e include effects on fatty acid synthesis from acetate by liver [*177*] effects on hepatic glycogen synthesis [*178*] effects on amino acid concentration [*179*] and incorporation into protein [*180*] by skeletal muscle effects on hepatic glucose release [*181 182*] and general effects on permeability of cell membranes for substances as different from glucose as the enzyme aldolase [*183*] It has also been suggested that in skeletal muscle insulin primarily affects electric membrane potentials and ionic concentration gradients [*184*]

Tissues vary markedly in their sensitivity to insulin Recently it has been shown that adipose tissue may be more sensitive to small concentrations of insulin and to smaller changes in insulin concentrations than many other tissues which have been assayed in vitro [*185*] Table 3 2 summarizes some of the findings relating to the action of insulin on individual tissues It is obvious also from this table that certain tissues such as brain are apparently not affected by the presence or absence of insulin although this does not preclude effects on specific structures of small bulk within these tissues

## DIABETIC KETOACIDOSIS

Since the primary effect of insulin or at least one of the primary effects is to facilitate the utilization of glucose by certain tissues especially adipose tissue and muscle lack of insulin results in elevated extracellular fluid glucose levels Furthermore the altered hepatic enzyme structure characteristic of the diabetic state namely decreased glucokinase and increased glucose 6-phosphatase activity aggravates this tendency to extracellular accumulation of glucose The resulting hyperglycemia tends to correct *in part* for the defect in glucose utilization and there has been much debate as to whether this readjustment may compensate for the lack of insulin In general in the absence of ketoacidosis it would appear that such a compensation does indeed occur in the diabetic organism although it is generally assumed that this ability to maintain compensation by hyperglycemia is associated with states of partial rather than complete deficiency of the hormone

*In terms of over all carbohydrate balance a major part of the problem which faces the diabetic patient results from the occurrence of massive glucosuria as soon as the hyperglycemia surpasses the renal reabsorptive*

*capacity for glucose* As long as insulin deficiency is not complete compensation may occur with minimal to moderate hyperglycemia and thus minimal glucosuria With increasing severity of the insulin deficiency, higher and higher concentrations of glucose are required to achieve compensation and the effectiveness of this increased blood glucose level is to an increasingly large extent wasted by the occurrence of rapidly mounting urinary losses Still higher levels of blood glucose can be obtained only at the metabolic cost of profuse hepatic overproduction It is evident that peripheral underutilization and hepatic overproduction of glucose are intimately related in severe states of insulin deficiency

Excessive hepatic gluconeogenesis entails the concomitant catabolism of fatty acids and protein fatty acids to provide the necessary hydrogen for reduction of pyruvate to glucose protein to contribute carbon i e the pyruvate itself either directly or indirectly Since acetate is unable to contribute a *net* amount of carbon to the reversed glycolytic sequence fatty acids are unable to contribute carbon for gluconeogenesis Since urea is the major end product of nitrogen metabolism and since acetoacetate is the major intermediary product of hepatic catabolism of fatty acids it follows from the considerations of intermediary metabolism outlined above that the synthesis of one molecule of glucose from two molecules of pyruvate should be accompanied by the accumulation of at least one molecule of acetoacetate and one molecule of urea if the pyruvate was derived from amino acids

In terms of these concepts persons with mild diabetes with minimal glucosuria (of the order of 5 to 10 gm glucose per day) should not develop ketoacidosis since the excess hepatic catabolism of fatty acids required by this glucose loss does not produce more acetoacetate than can be easily metabolized by the organism On the other hand diabetic patients who lose 100 to 200 gm glucose per day necessarily catabolize a proportional amount of fatty acids and generate large amounts of ketone bodies When the production of ketone bodies surpasses the ability of the body to utilize them they accumulate in the blood stream and since their renal threshold is minimal an increasing number is lost in the urine Since acetoacetate and β hydroxybutyrate have relatively low pK values they are predominantly excreted as anions in conjunction with cations Thus sodium and potassium are lost further aggravating the osmotic diuresis already induced by the urinary glucose loss as well as the already existing tendency to metabolic acidosis

What are the mechanisms whereby the liver obtains the necessary fatty and amino acids for glucose synthesis? Let us first consider relatively recent information concerning adipose tissue metabolism During carbohydrate deprivation whether due to an absolute glucose lack such as in fasting or in hypoglycemia or to a relative lack such as occurs with insulin deficiency this tissue releases nonesterified fatty acids into the

circulation which bound to albumin are carried to the liver These fatty acids also serve as metabolic fuels which may be directly utilized by other tissues particularly heart and skeletal muscle Determination of nonesterified fatty acids in patients with diabetes has shown them to be at least as responsive to insulin as the concentration of blood glucose [186]

Turning to protein insulin has been shown to increase the incorporation of amino acids into muscle both in vivo and in vitro In the absence of insulin and/or carbohydrate the steady state of protein synthesis and breakdown from and to amino acids is altered in favor of protein breakdown Amino acids rise in the circulation and their hepatic metabolism appears to be roughly proportional to their concentration in blood Thus both for fatty acids from adipose tissue and for amino acids from muscle deposition or mobilization may be controlled at least in part by availability of insulin, or glucose or both

Recently several investigators have suggested that insulin also *directly* decreases hepatic glucose release [181 182] although their interpretations have been challenged [187] The metabolic effects of such a direct interference with hepatic gluconeogenesis would very likely resemble in all points the sequence of events described here Again as has so frequently been true during the development of endocrine metabolic concepts the controversy does not concern the occurrence of metabolic events but rather the relative importance assigned to individual events both in terms of quantity and in terms of their proximity to the primary site of action of hormones

In this discussion of the physiologic and biochemical induction of the acute diabetic syndrome no mention has been made of the role played by other endocrines notably the adrenal and pituitary The amelioration ' of the diabetic state by hypophysectomy or by adrenalectomy is fully established It would also seem that cortisone accelerates the efflux of amino acids from muscle [188] and perhaps facilitates their uptake into liver cells [179] Likewise corticosteroids and possibly pituitary factors such as ACTH or growth hormone accelerate the release of unesterified fatty acids from adipose tissue It would appear therefore that the adrenal and pituitary glands oppose the effects of insulin on muscle and adipose tissue with regard to amino acid and fatty acid balance respectively From this it would follow that adrenalectomized or hypophysectomized animals unable to mobilize glucose precursors from the periphery should show decreased gluconeogenesis and therefore less nitrogen loss and a decreased predisposition to ketosis It should be stressed however that the diabetic adrenalectomized or diabetic hypophysectomized state implies loss of the ability to adapt to either hyperglycemia or hypoglycemia and as the clinician well knows may be more difficult to manage than either endocrinopathy alone

*Nondiabetic Ketoacidosis*

That glucosuria is the prime instigator of the entire sequence of events leading to ketoacidosis is corroborated by observations in two other metabolic states characterized by loss of carbohydrate from the body The first of these is *the experimental animal in which primary renal loss of glucose has been induced by phlorizin* and which exhibits a sequence of chemical anomalies identical to that noted in diabetes with the exception of hyperglycemia which is replaced by hypoglycemia Occasionally hereditary renal glucosuria in man may be of such degree that mild ketoacidosis occurs The second metabolic state to be mentioned here is *bovine ketosis* i e carbohydrate loss in milk a condition frequently seen in cows during active lactation Affected animals usually the optimal milk producers in the herd exhibit classical ketoacidosis with Kussmaul respirations dehydration and collapse Again hypoglycemia is present rather than hyperglycemia and indeed therapy dramatically effective consists of the intravenous administration of large amounts of 50 per cent glucose! Finally another and well known cause of mild ketoacidosis is the excessive utilization of carbohydrate in the body itself as in severely ill or febrile patients particularly children Again the metabolic sequence is excessive hepatic gluconeogenesis requiring simultaneous catabolism of fatty acid and protein

## SUMMARY AND CONCLUSIONS

1 Diabetes mellitus may be defined as a metabolic disorder characterized by hyperglycemia Although hyperglycemia is not present from birth in the great majority of instances there is excellent evidence to indicate that the potentiality to develop diabetes mellitus is inherited Best available evidence suggests that the mode of inheritance is a simple recessive one with incomplete penetrance

2 Genealogies which do not seem consistent with the simple recessive mode of inheritance may be the result of the very high incidence of the diabetic gene combined with inability to recognize its presence prior to the development of hyperglycemia It has been estimated that in the United States about 5 per cent of the population is homozygous (dd) for the gene determining the susceptibility for diabetes Available information does not suggest that the major clinical forms of diabetes are genetically distinct the rare syndrome of lipoatrophic diabetes being a probable exception

3 The diabetic manifestations which are more or less readily explained as derived from hyperglycemia have been termed *acute diabetic syndrome* while the *chronic diabetic syndrome* further includes manifestations which develop slowly in the course of the disease and which frequently appear

to have a vascular basis Evidence derived from the study of experimental diabetes in animals as well as from careful measurements of the mass of the islets of Langerhans in patients suffering from diabetes suggests that the acute diabetic syndrome is the result of absolute or relative deficiency of the hormone produced by the beta cells of the islets of Langerhans i e insulin All the symptoms and signs of the acute diabetic syndrome may be reversed by the administration of insulin Whether absolute or relative insulin deficiency also accounts for the symptoms and signs of the chronic diabetic syndrome is as yet uncertain

4 Whereas absolute or relative insulin deficiency is indeed likely to be an important pathogenetic link in the production of the acute diabetic syndrome the mechanism producing this deficiency is as yet completely unknown Possibilities which have to be considered include the mechanisms of insulin synthesis storage release and secretion transport destruction and effectiveness at the tissue level In contrast a great deal of information has accumulated with regard to the pathogenetic pathways linking the acute diabetic syndrome to the postulated insulin deficiency this information has been derived from the study of animals with experimental forms of diabetes of diabetic patients and of the action of insulin in the intact organism and in isolated tissues Much evidence points to the importance of insulin in facilitating the transport and/or metabolic activation of glucose Indeed most features of the acute diabetic syndrome can be explained as secondary results of decreased glucose utilization by certain tissues among which adipose tissue and muscle probably are of particular importance The metabolic interrelations upon which this summary statement is based have been discussed in some detail with emphasis upon the fact that the metabolism of carbohydrates fats and proteins should not be considered separately but as completely and intimately interrelated systems

## BIBLIOGRAPHY


1 Allen F M *Studies Concerning Glycosuria and Diabetes* Leonard Boston 1913
2 Allen F M Stillman E and Fitz R *Total Dietary Regulation in the Treatment of Diabetes* Monograph 11 Rockefeller Institute for Medical Research New York 1919
3 Jensen H F *Insulin Its Chemistry and Physiology* Commonwealth Fund New York 1938
4 Dobson M Experiments and observations on the urine in diabetes Medical Observations and Inquiries 5 298 1776
5 Joslin E P Root H F White P and Marble A *Treatment of Diabetes Mellitus* 10th ed Lea & Febiger Philadelphia 1959
6 Friedenwald J Diabetic retinopathy Am J Ophth **33** 1187 1950
7 LeCompte P M Vascular lesions in diabetes mellitus J Chron Dis **2** 178 1955
8 Warren S and LeCompte P M *The Pathology of Diabetes* p 170 Lea & Febiger Philadelphia 1959

9 Wilkerson H L C and Krall L P Diabetes in New England town study of 3 516 persons in Oxford Mass J A M A 135 209 1947
10 Wilkerson H L C and Krall L P Diabetes in New England town report of 4 years progress study of Oxford Mass diabetes survey of 1946–1947 J A M A 152 13 1953
11 Wilkerson H L C and Krall L P Follow up study cited by Joslin E P et al in *Treatment of Diabetes Mellitus* 10th ed p 36 Lea & Febiger Philadelphia 1959
11a Remein Q R A current estimate of the prevalence of diabetes mellitus in the United States Ann N Y Acad Sc 82 229 1959
12 Ponteva E Über die Resultate der Diabetesbehandlung in Finland Acta med scandinav Suppl 88 1 1938
13 White P Evidence in favor of the theory that diabetes is inherited in *Treatment of Diabetes Mellitus* edited by E P Joslin et al 10th ed p 48 Lea & Febiger Philadelphia 1959
14 Hanhart E Neue Forschungs-ergebnisse über die Vererbung des Diabetes Mellitus Arch Julius Klaus-Stift 25 586 1950
15 Hanhart E Zur Vererbung des Diabetes Mellitus Schweiz med Wchnschr 81 1127 1951
16 Hanhart E Die erbliche Anlage der Zuckerkrankheit heutiger Stand Deutsche Ztschr Verdauung kr 13 145 1953
17 Then Bergh H The genetic aspect of diabetes mellitus Foreign Letters Berlin J A M A 112 1091 1939 and Die Erbbiologie des Diabetes Vorläufiges Ergebnis der Zwillings-untersuchungen Arch Rass.enbiol 32 289 1938
18 White P in *Treatment of Diabetes Mellitus* 10th ed p 49 Lea & Febiger Philadelphia 1959
19 Guest G M and Warkany J Twins and diabetes mellitus Diabetes 5 150 1956
20 Steinberg A G Heredity and diabetes Diabetes 7 244 1958
21 von Kries I Beitrag zur Genetik des Diabetes Mellitus Ztschr menschl Vererb u Konstitutionslehre 31 406 1953
Levit S G and Pessikova L N Proc Maxim Gorky Medico-Biol Inst Moscow 3 132 1934
23 Penrose L S and Watson E M Sex linked tendency in familial diabetes Proc Am Diabetes A 5 163 1945
24 Hoet J P *Diabetes and Pregnancy* Extrait des *Acquisitions médicales récentes* Éditions médicales Flammarion 1953
25 Ditzel J White P and Sargeant L A follow up study of children of young diabetic mothers Acta genet et statis med 7 101 1957
26 Steinberg A G Inheritance and anticipation in diabetes mellitus in relation to carbohydrate metabolism during pregnancy Diabetes 4 126 1955
27 Jackson W P U The prediabetic syndrome Large babies and the (pre) diabetic father J Clin Endocrinol 14 177 1954
28 Van Beek C C Kan men aan een doodgeborene de diagnose diabetes mellitus der moeder stellen? Nederl tijdschr geneesk 83 5973 1939
29 Woolf N and Jackson W P U Maternal prediabetes and the foetal pancreas J Path & Bact 74 223 1957
30 Okamato K Experimental pathology of diabetes mellitus II Tohoku J Exper Med 61 Suppl III 1 1955
31 Bartelheimer H and Kloos K Die Auswirkung des experimentellen Diabetes auf Gravidität und Nachkommenschaft Ztschr ges exper Med 119 246 1952
32 Hoet J P Carbohydrate metabolism during pregnancy Diabetes 3 1 1954
33 Wilkerson H L C and Remein Q R Studies of abnormal carbohydrate metabolism in pregnancy The significance of impaired glucose tolerance Diabetes 6 324 1957
34 Pedersen J *Diabetes and Pregnancy Blood Sugar of Newborn Infants* Danish Science Press Copenhagen 1952
35 Pyke D A Parity and diabetes Lancet 2 818 1956

36 Steinberg A G Genetics of diabetes Ann N Y Acad Sc 82 197 1959
37 Knowles H C The incidence and devel pment of diabetes mellitu in *Diabetes* edited by R H William p 360 Hoeber New York 1960
38 Harris H Familial distribution of diabetes mellitus study of relatives of 1241 diabetic propositi Ann Eugenics 15 95 1950
39 Cammidge I J Heredity as a factor in the aetiology of diabetes mellitus Lancet 1 393 1934
40 Steinberg A G and Wilder R M An analysis of the phenomen n of antic ipation in diabetes mellitus Ann Int Med 36 1?8 1952
41 Steinberg A G and Wilder R M A study of the genetics of diabetes mellitus Am J Human Genet 4 113 1952
4 Steinberg A G Heredity and diabetes Eugenics Quarterly 2 2? 1???
43 Ingalls A M Dickie M M and Snell G D Obese new mutation in house mouse J Hered 41 317 1950
44 Wrenshall G A Andrus S B and Mayer J High levels of pancreatic insulin coexistent with hyperpl sia and degranulation of beta cells in mice with heredi tary obese hyperglycemia syndrome Endocrinology 56 335 1955
45 Christophe J Dagenais Y M and Mayer J Increased circulating insulin like activity in obese hyperglycemic mice Nature London 184 61 1959
46 Mayer J and Silides D J Fat metabolism in experimental obesities VIII Blood total lipids and ketones in four kinds of obese mice Experientia 14 96 1958
47 Yerganian G The striped back or chinese hamster J Nat Cancer Inst 20 705 1958
48 Meier H and Yerganian C A Spontaneous hereditary diabetes mellitus in chinese hamster 1 Pathological findings Proc Soc Exper Biol & Med 100 810 1959
49 Zahnd G Renold A E and Yerganian G A Unpublished observations
50 Argyris B F The mechanism of insulin resistance in the KL strain of mice inactivation of insulin Endocrinology 64 400 1959
51 Conn J W The prediabetic state in man definition interpretation and implica tions Diabetes 7 347 1958
51a Fajans S S and Conn J W The early recognition of diabetes mellitus Ann N Y Acad Sc 82 208 1959
52 Kriss J I and Futcher P H The relation between infant birthweight and subsequent development of maternal diabetes mellitus J Clin Endocrinol 8 380 1948
53 Mehring J von and Minkowski O Diabetes mellitus nach Pankreasexstirpa tion Arch exper Path u Pharmakol 26 371 1890
54 Dunn J S Sheehan H L and McLetchie N G B Necrosis of the islets of Langerhans produced experimentally Lancet 1 484 1943
55 Patterson J W Diabetogenic effect of dehydroascorbic acid Endocrinology 45 344 1949
56 Kadota I and Tokuyoshi A Chemical specificity of diabetogenic action of quinoline derivatives J Lab & Clin Med 43 375 1954
57 Moloney P J and Coval M Antigenicity of insulin diabetes induced by specific antibodies Bio hem J 59 179 1955
58 Wright P H Production of acute insulin deficiency by administration of insulin antiserum Nature London 183 8 9 1959
59 Young F G Permanent experimental diabetes produced by pituitary (anterior lobe) injections Lancet 2 372 1937
60 Ketterer B Randle P J and Young F G The pituitary growth hormone and metabolic processes in *Ergebnisse der Physiologie Biologischen Chemie und experimentellen Pharmakologie* p 127 Springer Berlin 1957
61 Long C N H The influence of the adrenal cortex on carbohydrate metabolism in *Hormonal Factors in Carbohydrate Metabolism* p 136 Little Brown Boston 1953
62 Houssay B A Thyroid and diabetes Vitamins & Hormones 4 187 1946

63 Lukens F D W Dohan F C and Wolcott M W Pituitary-diabetes in the cat recovery following pl lori izin treatment Endocrinology 32 4/5 1943
64 Dohan F C and Lukens F D W Lesions of pancreatic islets produced in cats by administration of glucose Science 105 183 1947
65 Gepts W Contribution à l étude morphologique des ilots de Langerhans au cours du diabète Ann soc roy sci méd et nat Bruxelles 10 5 1957
66 Maclean N and Ogilvie R F Quantitative estimation of the pancreatic islet tissue in diabetic subjects Diabetes 4 1 1955
67 Wrenshall G A Bogoch A and Ritchie R C Extractable insulin of pancreas correlation with pathological and clinical findings in dial etic and nondial etic cases Diabetes 1 87 1952
68 Sanger F The chemistry of insulin Science 129 1340 1959
69 Sanger F Ann Rep on Progr Chem (Chem Soc London) 45 283 1948
70 Lindner F Chemie und Bioch emie des Insulins in *Insulin und Insi lin Therapie* p 17 Urban & Schwarzenberg Munich and Berlin 1956
71 Rothen A Chow B F Greep R O and van Dyke H B Specific properties of films of proteins Cold Spring Harbor Symposia Quant Biol 9 272 1941
72 Marks H P and Young F G Species variation in insulin content of pancreas Nature London 146 31 1946
73 Jorpes E and Rastgeldi S The insulin content of the human pancreas Acta pl ysiol Scand nav 29 163 1953
74 Maske H Interaction between insulin and zinc in the islets of Lang rhans Diabetes 6 335 1957
75 Maske H Beol achtungen über das Zink in den Langerhans cl en Inseln des I ankreas und seine Bezi l ungen zur Inselfunktion Ztschr Naturforsch 8b 96 1953
76 Lindner F Ueber ein genuines Depot Insulin Praparat (Nativ Insulin) Medizin un Chemie 4 248 1940
77 Hartroft W S and Wrensl all G A Correlation of beta-cell granulation with extractable insulin of the pancreas Diabetes 4 1 1955
77a Haist R E Islet cell function Ann N Y Acad Sc 82 206 1959
78 Batts A Use of the Golgi apparatus as an indicator of the level of activity of the cells of the islets of Langerhans Ann N Y Acad Sc 82 302 1959
79 Foá P P The control of the secretory activity of the islets of Langerhans in *Internal Secretions of the Pancreas* vol 9 p 55 Little Brown Boston 1955
79a Gayet R and Guillaumie M La régulation de la glycémie des chiens diabétiques par des quantités variées de tissu pancréatique transplanté Compt rend soc biol 98 676 1928
80 Houssay B A Lewis J T and Foglia V G Action compensative ou préventive de la greffe pancréatique sur la glycemie diabétique ou normale Compt rend soc biol 100 140 1929
81 Brown E M Jr Dohan F C Freedman L R DeMoor P and Lukens F D W The effects of prolonged infu ion of the dog s pancreas with gluco e Endocrinology 50 644 1952
82 Hagedorn H C On protamine-splitting properties of blood-serum Scandinav Arch Pl ys ol 80 156 1938
83 Lazarus S S Acid and glucose 6-pho phatas activity of panc eati β cells Proc Soc Exper B ol & Med 102 303 1959
84 Duncan L J P and Baird J D Compounds admi ster l orally in the treatment of diabetes Pharmacol Rev 12 91 1960
85 Wallenfelds K Summ H D and Creutzfeldt W Enzymati che Unter suchungen zum Wirkungsmechani mus de l lutzuckersenkenden Medikamente Deutsche med Wchnschr 82 1581 1957
86 Beigelman P M Insul n like activity of serum protein fractions Diabetes 7 365 1958
87 Antoniades H N Beigelman P M and Renold A E in *Ho mones in Human Plasma* edited by H N Antoniades Little Brown Boston 1960
88 Goetz F C Beigelman P M and Thorn G W A metho l of insulin bio as av

and its application to human plasma fractions Proc Soc Expr Biol & Med 86 481 1954
89 Beigelman P M Antoniades H N Goetz F C Renold A E Oncley J L and Thorn G W Insulin like activity of human plasma constituents II Biologic assay of human plasma fractions for insulin like activity Metabolism 5 44 1956
90 Antoniades H N Separation of human plasma protein concentrate with insulin activity Science 127 593 1958
91 Welsh G W Henley E D Williams R H and Cox R W Insulin I 131 metabolism in man Plasma binding distribution and degradation Am J Med 21 324 1956
92 Berson S A Yalow R Bauman A Rothschild M and Newerly K Insulin $I^{131}$ metabolism in human subjects demonstration of insulin binding globulin in the circulation of insulin treated subjects J Clin Invest 35 170 1956
93 Lowell F C Immunologic studies in insulin resistance II The presence of a neutralizing factor in the blood exhibiting some characteristics of an antibody J Clin Invest 23 233 1944
94 Krahl M E in *Hormones in Human Plasma* edited by H N Antoniades Little Brown Boston 1960
95 Randle P in *Diabetes* edited by R H Williams (in press)
96 Field J B Tietze F and Stetten D Jr Further characterization of an insulin antagonist in the serum of patients in diabetic acidosis J Clin Invest 36 1588 1957
97 Bornstein J Insulin reversible inhibition of glucose utilization by serum lipoprotein fractions J Biol Chem 205 513 1953
98 Vallance Owens J and Lukens F D W Studies on insulin antagonism in plasma Endocrinology 60 625 1957
99 Mirsky I A The role of insulinase and insulinase inhibitors Metabolism 5 138 1956
100 Mirsky I A Perisutti G and Dixon F J The destruction of $I^{131}$ labeled insulin by rat liver extracts J Biol Chem 214 397 1955
101 Tomizawa H H and Williams R H Studies on the specificity of an insulin inactivating system of the liver J Biol Chem 217 685 1955
102 Williams R H Martin F B Henley E D and Swanson H E Inhibitors of insulin degradation Metabolism 8 99 1959
103 Stadie W C Haugaard N and Vaughan M Insulin binding with isotopically labeled insulin J Biol Chem 199 729 1952
104 Stadie W C Haugaard N and Vaughan M The quantitative relation between insulin and its biological activity J Biol Chem 200 745 1953
104a Lacey P E and Hartroft W S Electron microscopy of the islets of Langerhans Ann N Y Acad Sc 82 287 1959
105 Wrenshall G A and Best C H Extractable insulin of the pancreas and effectiveness of oral hypoglycemic sulfonylureas in the treatment of diabetes in man—a comparison Canad M A J 74 968 1956
106 Wrenshall G A The pancreas and diabetes mellitus in man and animals Minnesota Med 41 342 1958
107 Wrenshall G A and Hamilton J D Sex differences in the amount of insulin extractable from diabetic human pancreas Ann New York Acad Sc 71 154 1957
108 Bornstein J and Lawrence R D Plasma insulin in human diabetes mellitus Brit M J 2 1541 1951
109 Vallance Owen J Hurlock B and Pease M W Plasma insulin activity in diabetes mellitus Lancet 269 68 1955
110 Randle P J Insulin in blood in *Hormones in Blood* vol II p 115 Little Brown Boston 1957
111 Willebrands A F and Groen J Determination of serum insulin by the rat diaphragm method Further observations in diabetic and nondiabetic subject and in hyperinsulinism Diabetes 5 378 1956

112 Himsworth H P Insulin-sensitive and insulin insensitive types Lancet 1 127 1936
113 Lawrence R D Three types of human diabetes Ann Int Med 43 1199 1955
114 Field J B Symposium on disorders of carbohydrate metabolism on the nature of the metabolic defect(s) in diabetes Am J Med 26 662 1959
115 Lawrence R D Lipodystrophy and hepatomegaly with diabetes lipemia and other metabolic disturbances Lancet 250 724 1946
116 Schwartz R Schafer I A and Renold A E Generalized lipoatrophy hepatic cirrhosis disturbed carbohydrate metabolism and accelerated growth (lipoatrophic diabetes) longitudinal observation and metabolic studies Am J Med 1960
117 Craig J W and Miller M Lipoatrophic diabetes in *Diabetes* edited by R H Williams p 600 Hoeber New York 1960
118 Field J B Weinberg A Johnson I and Spoont S An unusual case of diabetes mellitus with increased pancreatic production of insulin J Clin Invest 38 1003 1959
119 Leonards J R and Martin F I R Insulin insensitivity a variant of insulin resistance New England J Med 261 68 1959
120 Banting F G and Best C H Pancreatic extracts J Lab & Clin Med 7 464 1922
121 Special article The status of insulin J A M A 80 1238 1923
122 Hepburn J and Latchford J K Effect of insulin (pancreatic extract) on the sugar consumption of the isolated surviving rabbit heart Am J Physiol 62 177 1922
123 Knowlton F P and Starling E H Experiments on the consumption of sugar in the normal and the diabetic heart J Physiol 45 146 1912
124 Clark A H The interrelation of the surviving heart and pancreas of the dog in sugar metabolism J Exper Med 24 621 1916
125 Cori C F Cori G T and Goltz H C Blood sugar concentration in liver vein leg artery and leg vein during insulin action J Pharmacol & Exper Therap 22 355 1923
126 Mann F C and Magath T B Studies on physiology of liver effect of insulin on blood sugar following total and partial removal of liver Am J Physiol 65 403 1923
127 Burn J H and Dale H H On the location and nature of the action of insulin J Physiol 55 164 1924
128 Kellaway C H and Hughes T A Observation on the influence of insulin on normal metabolism in man Brit M J 1 710 1923
129 Sutherland E W and Cori C F Effect of hyperglycemic glycolytic factor and epinephrine on liver phosphorylase J Biol Chem 188 531 1951
130 Sutherland E W and Rall T W Fractionation and characterization of a cyclic adenine ribonucleotide formed by tissue particles J Biol Chem 232 1077 1958
131 Harden A and Young W J The function of phosphate in the fermentation of glucose by yeast juice Proc Roy Soc London S B 80 299 1908
132 Embden G and Laquer F Über die Chemie des Lactacidogens Ztschr physiol Chem 113 1 1921
133 Meyerhof O Die Milchsaurebildung aus den garfähigen Hexosen Biochem Ztschr 183 176 1927
134 Ashmore J and Weber G Glucose-6-phosphatase Vitamin & Hormones 17 92 1959
135 Hers H G Berthet J Berthet L and deDuve C Le système hexose phosphatasique III Localisation intracellulaire des ferments par centrifugation fractionée Bull Soc chim biol 33 21 1951
136 Cori G T and Cori C F The kinetics of the enzymatic synthesis of glycogen from glucose-1-phosphate J Biol Chem 135 733 1940
137 Trevelyan W L Mann P F E and Harrison J S The phosphorylase

reaction III The glucose-1 phosphate and orthophosphate content of Baker's yeast in relation to polysaccharide synthesis Arch Biochem 60 81 1954
138 Larner J Villar Palasi C and Richman D J Insulin stimulated glycogen formation in rat diaphragm Ann New York Acad Sc 82 345 1959
139 Sutherland E W Jr Hormonal activation of phosphorylase in *Enzymes Units of Biological Structure and Function* p 536 Henry Ford Hospital Academic Press Inc New York 1956
140 Cahill G F Jr Ashmore J Zottu S and Hastings A B Studies on carbohydrate metabolism in rat liver slices IX Ionic and hormonal effects on phosphorylase and glycogen J Biol Chem 224 237 1957
141 Beloff Chain A Catanzaro R Chain E B Masi I Pocchiari F and Rossi C The influence of insulin on carbohydrate metabolism in the isolated diaphragm muscle of normal and alloxan diabetic rats Proc Roy Soc London S B 143 481 1955
142 Leloir L F and Cardini C E Biosynthesis of glycogen from uridine diphosphate glucose J Am Chem Soc 79 6340 1957
143 Cori G T Glycogen structure and enzyme deficiencies in glycogen storage disease Harvey Lect 48 145 1954
144 Stetten M R and Stetten DeW Jr Glycogen regeneration in vivo J Biol Chem 213 723 1955
145 Olson R E Oxidation of $C^{14}$ labeled carbohydrate to intermediates in tumor and normal tissues Cancer Res 11 571 1951
146 Mokrasch L C Davidson W D and McGilvery R W The response to glucogenic stress of fructose 1 6-diphosphatase in rabbit liver J Biol Chem 222 179 1956
147 Horecker B L and Hiatt H H Pathways of carbohydrate metabolism in normal and neoplastic cells New England J Med 258 177 225 1958
148 Wakil S J A malonic acid derivative as an intermediate in fatty acid synthesis J Am Chem Soc 80 6465 1958
149 *Ciba Foundation Symposium on the Biosynthesis of Terpenes and Sterols* Little Brown Boston 1959
150 Bucher N L R McGarrahan K Gould L and Loud A V Cholesterol biosynthesis in preparations of liver from normal fasting X irradiated cholesterol fed triton or $\Delta^4$-cholesterol-3-one treated rats J Biol Chem 234 26 1959
151 Lynen F Henning U Bublitz C Sorbo B and Kroplin Rueff L Der chemische Mechanismus der Acetessigsäurebildung in der Leber Biochem Ztschr 330 269 1958
152 Krebs H A Considerations concerning the pathways of synthesis in living matter synthesis of glycogen from non-carbohydrate precursors Bull Johns Hopkins Hosp 95 19 1954
153 Krimsky I Phosphorylation of pyruvate by the pyruvate kinase reaction and reversal of glycolysis in a reconstructed system J Biol Chem 234 232 1959
154 Siperstein M D Inter relationships of glucose and lipid metabolism Am J Med 26 685 1959
155 Villee C A and Hagerman D D Effects of estradiol on metabolism of human placenta in vitro J Biol Chem 205 873 1953
156 Soskin S Essex H E Herrick J P and Mann F C The mechanism of regulation of the blood sugar by the liver Am J Physiol 124 558 1938
157 Cahill G F Jr Ashmore J Renold A E and Hastings A B Blood glucose and the liver Am J Med 26 64 1959
158 Ashmore J Cahill G F Jr Hastings A B and Zottu S Studies on carbohydrate metabolism in rat liver slices VIII Effect of ions and hormones on pathways of glucose 6-phosphate metabolism J Biol Chem 224 225 1957
159 Renold A E Hastings A B and Nesbett F B Studies on carbohydrate metabolism in rat liver slices III Utilization of glucose and fructose by liver from normal and diabetic animals J Biol Chem 209 687 1954
160 Siperstein M D and Fagan V M Studies on the relationship between

glucose oxidation and intermediary metabolism I The influence of glycolysis on the synthesis of cholesterol and fatty acid in normal liver J Clin Invest 37 1185 1958
161 Glock G E and McLean P Levels of enzymes of direct oxidative pathway of carbohydrate metabolism in mammalian tissues and tumors Biochem J 56 171 1954
162 Hiatt H H Goldstein M Larcau J and Horecker B L The pathway of hexose synthesis from pyruvate in muscle J Biol Chem 231 303 1958
163 Andres R Cader G and Zierler K L The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm J Clin Invest 35 671 1956
164 Favarger P and Gerlach J Recherches sur la synthèse des graisses à partir d acétate ou de glucose II Les rôles respectifs du foie du tissu adipeux et de certains autres tissus dans la lipogenèse chez la souris Helvet physiol et pharmacol acta 13 96 1955
165 Winegrad A I and Renold A E Studies on rat adipose tissue *in vitro* II Effects of insulin on the metabolism of specifically labeled glucose J Biol Chem 233 273 1958
166 Bragdon J H and Gordon R S Jr Tissue distribution of C[14] after the intravenous injection of labeled chylomicrons and unesterified fatty acids in the rat J Clin Invest 37 574 1958
167 Fredrickson D S and Gordon R S Transport of fatty acids Physiol Rev 38 585 1958
168 White J E and Engel F L A lipolytic action of epinephrine and norepinephrine on rat adipose tissue *in vitro* Proc Soc Exper Biol & Med 99 375 1958
169 Colowick S P Cori G T and Slein M W The effect of adrenal cortex and anterior pituitary extracts and insulin on the hexokinase reactions J Biol Chem 168 583 1947
170 Levine R Goldstein M Huddlestun B and Klein S Action of insulin on the permeability of cells to the hexoses as studied by its effect on the distribution of galactose Am J Physiol 163 70 1950
171 Ross E J The permeability hypothesis of the action of insulin Medicine 35 355 1956
172 Stadie W C and Haugaard N The hexokinase reaction in tissue extracts *from normal and diabetic rats J Biol Chem 177 311 1949*
173 Morgan H E Henderson M V Regen D M and Park C R The regulation of glucose uptake in heart muscle from normal and alloxan diabetic rats the effects of insulin growth hormone cortisone and anoxia Ann New York Acad Sc 82 387 1959
174 Kipnis D M Regulation of glucose uptake by muscle functional significance of permeability and phosphorylating activity Ann N Y Acad Sc 82 354 1959
175 Park C R Reinwein D Henderson M V Cadenas E and Morgan H E The action of insulin on the transport of glucose through the cell membrane Am J Med 26 679 1959
176 Cahill G F Jr Ashmore J Earle A S and Zottu S Glucose penetration into liver Am J Physiol 192 491 1958
177 Brady R O Lukens F D W and Gurin S Synthesis of radioactive fatty acids *in vivo* and its hormonal control J Biol Chem 193 959 1951
178 Bertlet J Jaques P Hers H G and de Duve C Influence de l insuline et du glucagon sur la synthèse du glycogène hépatique Biochim et biophys acta 20 190 1956
179 Noall M W Riggs T R Walker L M and Christensen H N Endocrine control of amino acid transfer Science 126 1002 1957
180 Wool I G and Krahl M E Incorporation of C amino acids into protein of isolated diaphragms an effect of insulin independent of glucose entry Am J Physiol 196 961 1959

181 Madison L L Combes B Strickland W Unger R and Adams R Evidence for a direct effect of insulin on hepatic glucose output Metabolism 8 469 1959
182 Reichard G A Jacobs A C Friedmann B Kimbel F R Hochella N J and Weinhouse S Effects of insulin and tolbutamide on production and utilization of blood sugar Metabolism 8 486 1959
183 Zierler K L Increased muscle permeability to aldolase produced by insulin and albumin Am J Physiol 192 283 1958
184 Zierler K L Increase in resting membrane potential of skeletal muscle produced by insulin Science 126 1067 1957
185 Renold A E and Winegrad A I Insulin action effects on individual tissues in *Diabetes* edited by R H Williams p 129 Hoeber New York 1960
186 Bierman L L Dole V P and Roberts T N An abnormality of nonesterified fatty acid metabolism in diabetes mellitus Diabetes 6 475 1957
187 Shoemaker W C Mahler R and Ashmore J The effect of insulin on hepatic glucose metabolism in the unanesthetized dog Metabolism 8 494 1959
188 Bondy P K Effect of adrenal and thyroid glands upon rise of plasma amino acids in eviscerated rat Endocrinology 45 605 1949

# Chapter 4

## Pentosuria*

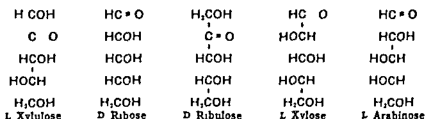
*Howard H Hiatt*

The first six decades which followed the original description by Salkowski and Jastrowitz in 1892 of an individual with essential pentosuria [1] produced much information concerning the clinical aspects of this condition but virtually none which cast light on the nature of the biochemical lesion Before the seventh decade has elapsed however one can predict with confidence that the metabolic defect will have been well characterized As a result and perhaps even more significantly important information will have been uncovered concerning the operation in normal individuals of a previously unknown pathway of carbohydrate metabolism Such an elucidation of a normal mechanism by studies of an accident of nature led Garrod [2] 30 years ago to stress the lessons of rare maladies and prompted Harvey [3] 300 years earlier to note that nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path

A consideration of essential pentosuria requires its separation from other conditions in which a five carbon sugar is excreted in the urine Essential pentosuria or chronic essential pentosuria is the only member of the group in which a genetic defect accounts for the melituria It may be further defined as an innocuous condition presumably present from birth in which a relatively constant amount of the pentose L-xylulose appears in the urine It bears no relationship to diabetes mellitus and has been described almost exclusively in Jews It is almost surely the result of an impairment in the metabolism of glucuronic acid It may easily be distinguished from several other situations in which much smaller quantities of certain pentoses other than L-xylulose are found

* This work was supported by grants from the National Cancer Institute Public Health Service and the Jane Coffin Childs Memorial Fund for Medical Research

in the urine (Table 4.1). The structure of some of the urinary pentoses follows:




Alimentary pentosuria is the term applied to the excretion of small amounts of arabinose or xylose[1] following the ingestion of unusually large quantities of such fruits as plums, cherries, and grapes, and fruit juices [4–6]. Small amounts of D-ribose are often present in urine from healthy

TABLE 4.1 TYPES OF PENTOSURIA

| *Type* | *Urine pentose* | *Amount excreted gm/24 hr* | *Cause* | *Origin of pentose* |
|---|---|---|---|---|
| Essential | L-Xylulose | 1.0–4.0 | Metabolic error | D-glucuronic acid |
| Alimentary | L-Arabinose<br>L-Xylose | Less than 0.100 | Excessive fruit intake | Dietary fruit |
| Ribosuria | D-Ribose | Up to 0.030 | Muscular dystrophy | Muscle coenzymes (?) |
| Normal | L-Xylulose<br>D-Ribose<br>D-Ribulose | Up to 0.060<br>Up to 0.015<br>Traces | | |

persons, and slightly larger quantities may be found in the urine of some patients with muscular dystrophy [7], presumably as a result of the excessive breakdown of ribose-containing nucleotides in degenerating muscle. Finally, traces of L-xylulose [8, 9] and of D-ribulose [9] may be found in the urine of normal individuals. It is essential pentosuria with which this review will be primarily concerned.

## HISTORICAL SURVEY

In 1887 Kiliani [10] demonstrated that arabinose, the sugar of gum arabic, was a member of a hitherto undescribed class of sugars containing, in contrast to the hexoses, only five carbon atoms. Shortly thereafter the wood sugar, xylose, was identified as another member of this group.

[1] Although L-arabinose and L-xylose are said to be the alimentary pentoses [4, 5], the evidence for this chemical identification is not convincing.

The biologic significance of this class of sugars was strengthened by the report by Salkowski and Jastrowitz of a human being in whose urine a pentose was consistently excreted [1] This sugar was not fermented by yeast was optically inactive and yielded an osazone with a melting point of 159 C The latter observation led these authors to suggest that the sugar was a pentose Several similar case reports appeared in the German literature during the next decade and in 1906 Janeway [11] in a paper entitled Essential Pentosuria in Two Brothers recorded the first instances in an American publication Although Janeway acknowledged that in many of the previous reports in the literature neurasthenic symptoms were prominent he stressed that no harmful effects of the disorder were known He emphasized that the most important responsibility of the physician was to explain carefully to the patient the difference between his ailment and diabetes mellitus and to effect ' the removal of any dietetic restrictions he may have been subjected to Although more than a half century has elapsed since Janeway s report his advice still points out an essential function of any physician confronted by a person with pentosuria Two years after Janeway s paper in his classic Croonian lectures of 1908 Garrod [12] reviewed 30 recorded cases of essential pentosuria and he assigned this abnormality along with cystinuria alcaptonuria and albinism to a category which he labeled as inborn errors of metabolism He stressed the differences between essential pentosuria and the alimentary variety Garrod noted the incidence of essential pentosuria in Jews and the tendency for the condition to occur in several members of the same family A prevalent early impression that pentosuria frequently accompanies diabetes mellitus [13] has not been borne out

Much of the present knowledge concerning the clinical aspects of pentosuria its genetic transmission and the nature of the urinary sugar is derived from the careful observations of Enklewitz and Lasker [14-20] More than half of their 70 subjects with pentosuria were followed for periods in excess of 16 years Fifty years following the first description of pentosuria Derivaux [21] was able to collect 163 case reports from the literature and at the time of a recent review of the subject this figure exceeded 200 [22]

For many years controversy existed concerning the nature of the sugar in pentosuric urine The first chemical identification was that of Neuberg [23] who in 1900 reported that the sugar present in the urine of one case was racemic arabinose Five additional individuals with arabinosuria were subsequently described by Cammidge and Howard [24] and single similar cases were noted by a number of authors including Aron [25] Luzzatto [26] and Schüler [27] Zerner and Waltuch [28] in 1913 presented convincing evidence that the urinary pentose was optically active and that it was not arabinose The following year Levene and LaForge showed that

the urinary sugar in a case of pentosuria was L-xylulose[3] [29] Their identification was based on the following observations

1 An osazone of the urinary sugar had a melting point of 160 to 163°C when it was mixed with an osazone of D-xylose the melting point was increased by 10°

2 The initial optical rotation of the osazone was lower than the equilibrium rotation, this is characteristic of xylosazone but not of arabinosazone

3 The foregoing observations indicated that the urinary pentosazone was a xylosazone On the basis of the optical rotation of the urinary sugar ($\alpha_D{}^0 = +33.1°$) the character of its *p*-bromphenylhydrazone and its behavior in oxidation experiments Levene and LaForge concluded that the urinary pentose could only have been L-xylulose

In the same year Zerner and Waltuch [30] isolated the sugar from the urine of two patients with pentosuria and also concluded that it was L-xylulose A similar conclusion concerning the pentose in their cases was reached by Hiller [31] Greenwald [32] and Enklewitz and Lasker [15] Some of the early reports of arabinosuria were subsequently corrected For example a patient of Solis Cohen was first described in 1909 as having arabinosuria [33] At that time the author assumed from previously reported cases that arabinose was the urinary sugar excreted in pentosuria However the patient was followed for almost three decades and in a follow up report which appeared in 1936 [34] the same author indicated that he had identified the urinary sugar as L-xylulose and that his earlier impression had been in error Similarly a case reported as one of arabinosuria in 1913 [25] was reexamined 40 years later paper chromatography of his urine revealed the major sugar component to be L-xylulose and no evidence of arabinose was found [35]

The question of arabinosuria was considered at length in 1950 by Lasker [19] who indicated that she had identified L-xylulose in the urine of 72 individuals with pentosuria but had never seen a patient with arabinosuria She further stated that no case of arabinosuria had been reported since 1928 and that since in early reports the arabinose was always identified by the same method this impression may have been in error Recent studies with paper chromatographic techniques provide further corroboration that L-xylulose is the only sugar excreted in substantial amount in pentosuria In addition to L-xylulose a small quantity of L-arabitol has been isolated from the urine of one pentosuric patient [36] other reports that pentosuric patients excrete more than one pentose [35, 37] will be considered below

[3] The early reports of D xyloketose excretion were published prior to the adoption of the current practice of classifying sugars according to their structural relation to D and L-glyceraldehyde Throughout this review the present convention will be followed regardless of the terminology applied in the original reports

The constancy of the amount of urine pentose excreted by individuals with pentosuria has long been recognized Enklewitz and Lasker [16] found that the excretion in five adults varied from 1 1 to 3 7 gm per 24 hr but that the daily variation in any given subject never exceeded 0 9 gm The excretion is independent of dietary variations early reports by Janeway [11] and Klercker [13] that the amount of urinary pentose can be altered by changes in dietary nucleic acid or protein have not been substantiated Margolis noted a marked increase in pentose excretion in pentosuric subjects following aminopyrine ingestion [38] When Enklewitz and Lasker observed a similar stimulation not only following the intake of certain other drugs including borneol antipyrine and menthol but also after the administration of glucuronic acid [16] they ascribed the effect of the drugs to their glucuronogenic action The metabolic interrelations of glucuronic acid and L-xylulose however remained obscure and there was no experimental evidence for Everett s postulate that an abnormal enzyme system existed in pentosuric patients which decarboxylated glucuronic acid to L-xylulose [39] With the recent identification of L-xylulose as an intermediate in the metabolism of glucuronic acid [40 41] and the elucidation of the reactions involved in the further metabolism of L-xylulose [42 43] much progress has been made toward an understanding of the biochemical abnormality in pentosuria Much of the present concept stems from the careful studies of Touster and his associates [40–43] Further important contributions have come from the laboratories of Ashwell [44–46] Burns [47] Lehninger [48] and others Not only have these recent studies permitted an insight into the biochemical aberration in pentosuria but they have also provided important information concerning the operation of the glucuronic acid oxidation pathway in normal individual

## PENTOSE METABOLISM IN MAN

Several five carbon sugars are known to be present in the human organism Some such as ribose and deoxyribose are present as part of more complex substances including the nucleic acids and certain coenzymes Other including D xylulose D ribulose and L-xylulose are intermediates in metabolic pathways and normally are not detectable in body fluids or are present only in trace quantity Finally there are those which are not known to be synthesized by man but which occasionally may be ingested and thereafter are excreted in the urine These include arabinose and xylose

In recent years much information has come to light concerning previously obscure pathways of carbohydrate metabolism (for a summary see [49]) Certain of these schemes involve pentoses as key intermediates A consideration of these pathways is essential to understanding of the

several varieties of pentosuria that have been described including essential pentosuria

## THE PENTOSE PHOSPHATE PATHWAY [50]

Ribose the sugar moiety found in all ribonucleic acids and several coenzymes may be synthesized from glucose by either the oxidative or

Fig 4-1 The pentose phosphate pathway (*By permission of B L Horecker and H H Hiatt [49]*)

the nonoxidative reactions of the pentose phosphate pathways (Fig 4 1) In the oxidative reactions glucose 6-phosphate is converted successively to 6-phosphogluconolactone 6-phosphogluconic acid and following oxidative removal of *its first carbon atom* ribulose 5-phosphate The

latter may be isomerized to ribose 5 phosphate In this series of reactions for every molecule of pento e pho phate synthesized from hexose phosphate two molecules of tripho phopyridine nucleotide (TPN) are reduced to TPNH Much evidence has accumulated indicating that the conver ion of the coenzyme from its oxidized to its reduced form is as important a function of these reactions as is pentose production [49] This conclusion is ba ed on the increasing evidence for the TPNH re quirement of a variety of reductive synthetic reactions and on observa tions which sugge t that the oxidative reactions of the pentose phosphate pathway are the principal means available to the cell for TPN reduction

Ribose may also be produced nonoxidatively from hexo e phosphate via the transketolase and transaldolase reactions In these reactions the first two carbon atoms of one molecule of fructose 6-phosphate may be cleaved and condensed with a molecule of triose phosphate under the influence of the enzyme transketolase This results in the formation of one molecule of xylulo e 5-phosphate and one of erythrose 4 phosphate The latter together with another molecule of fructose 6-phosphate may then participate in the transaldolase reaction resulting in the production of a molecule of sedoheptulose 7 pho phate and one of triose pho phate In another reaction catalyzed by transketolase these products may undergo conversion to xylulose 5 phosphate and ribose 5-phosphate The xylulose 5 phosphate may be epimerized to ribulose 5 phosphate which as already noted can be isomerized to ribose 5 phosphate Thus in this series of reactions there is no net loss of carbon and two molecules of hexose phosphate and one of triose phosphate may be converted to three molecules of pentose phosphate Available data indicate that in animals [51 52] and in man [53] ribose is normally synthesized from hexo e by way of both the oxidative and the nonoxidative reactions of the pentose phosphate pathway The fact that thiamine pyrophosphate is a cofactor for the enzyme transketolase [54] accounts for the block in ribose synthe is by way of the nonoxidative sequence of reactions in the thiamine deficient animal [55]

In contrast to the oxidative reactions which afford only a mechanism for pentose production from hexose the transketolase transaldolase sequence is rever ible and provides a means for the interconversion of hexose and pentose [56] Evidence has been presented demonstrating that the nonoxidative reactions mediate the conversion of ribose to hexo e in man [57] It has also been shown that ribose may participate directly in riboside [55] and nucleic acid [58] synthesis Thus mechanisms exist for the disposition of any ribo e released in nucleic acid or coenzyme breakdown

Small quantities (up to 2 8 μmoles per 24 hr per kg body weight) of ribo e have been reported in normal human urine [7] This presumably repre ents either newly synthesized ribose or pentose released from

several varieties of pentosuria that have been described including essential pentosuria

## THE PENTOSE PHOSPHATE PATHWAY [30]

Ribose the sugar moiety found in all ribonucleic acids and several coenzymes may be synthesized from glucose by either the oxidative or

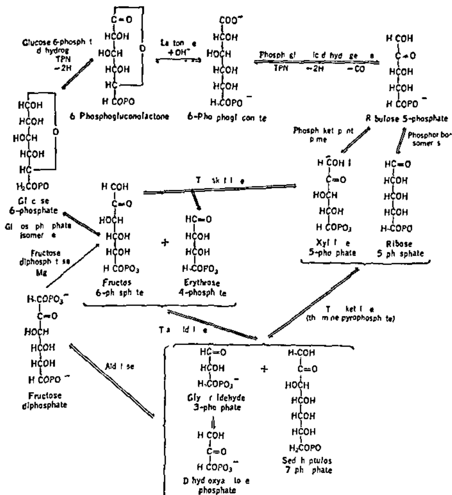



Fig 4 1 The pentose phosphate pathway (*By permission of B L Horecker and H H Hiatt* [29])

the nonoxidative reactions of the pentose phosphate pathway (Fig 4 1) In the oxidative reactions glucose 6-phosphate is converted successively to 6 phosphogluconolactone 6 phosphogluconic acid and following oxidative removal of its first carbon atom ribulose 5 phosphate The

The elucidation of the reactions involved in this pathway of metabolism provides insight not only into the origin of the L-xylulose found in trace amount in normal urine but also into the probable site of the defect in individuals with chronic essential pentosuria It also provides an explanation for the hitherto poorly understood increase in L-xylulose excretion by pentosuric subjects given glucuronic acid

The significance of the glucuronic acid oxidation pathway in human metabolism appears relatively minor This view is supported by the fact

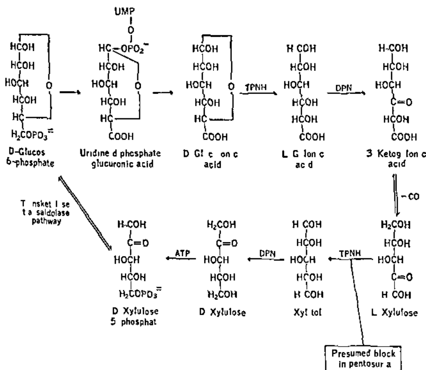



Fig 4 ? The glucuronic acid oxidation pathway

that individuals with pentosuria in whom this pathway is presumably blocked suffer no ill effects from the abnormality Although the reactions of the glucuronic acid oxidation pathway together with those of the pentose phosphate pathway provide a potential mechanism for ribose biosynthesis from hexose (i e hexose phosphate ⟶ glucuronic acid ⟶ xylulose ⟶ xylulose phosphate ⟶ ribose phosphate) animal studies indicate that little ribose is normally produced by way of this sequence of reactions [66] In most mammalian species the early reactions of the pathway are required for the production of L gulonic acid a precursor of ascorbic acid [67] In primates and the guinea pig however

nucleic acids or coenzymes which escapes further metabolism It has been asserted that ribose excretion is slightly but significantly increased in patients with myopathies presumably because of the increased breakdown of ribose containing compounds in diseased muscle [7] but this observation has not met universal acceptance [59] A metabolite of histamine imidazoleacetic acid is excreted by the rat [60 61] and by man [63] in part as the riboside Ribulose a trace constituent of normal urine [9] is presumably excreted following hydrolysis of the phosphate ester which is an intermediate in the pentose phosphate pathway

The mechanism involved in the synthesis of deoxyribose the sugar component of deoxyribonucleic acid has not yet been completely elucidated but at least a portion of it appears to be synthesized via direct reduction of the ribose molecule at the ribonucleotide level [62]

## THE GLUCURONIC ACID OXIDATION PATHWAY

The pentoses L-xylulose and D xylulose and the pentitol xylitol are intermediates in the glucuronic acid oxidation pathway (Fig 4 2) The reactions leading to the production of D glucuronic acid in vivo have not been completely elucidated but its carbon skeleton is known to originate in glucose [63 64] and the production of D glucuronic acid from uridine diphosphate glucuronic acid has recently been demonstrated in vitro [65] As has been noted the studies of Touster [40-43] Ashwell [44-46] Burns [47] and Lehninger [48] and their associates have provided much information concerning the further metabolism of D-glucuronic acid A reductive reaction involving TPNH as a cofactor results in the conversion of D glucuronic acid to L-gulonic acid L-Gulonolactone has been shown to be an intermediate in the synthesis of ascorbic acid in most animal species but in primates and in the guinea pig this transformation cannot take place L-Gulonic acid however may in all animal species examined be oxidized to the 3 keto derivative which in turn is decarboxylated to L-xylulose [46] In the latter reaction the atom corresponding to the sixth carbon atom of the parent glucose molecule is oxidized to $CO_2$ Carbon atom 1 of L-xylulose is derived from the fifth carbon atom of glucose Touster has prepared two enzymes from guinea pig liver one of which catalyzes the reduction of L xylulose to the sugar alcohol xylitol and the other of which promotes the oxidation of xylitol to D xylulose [42 43] These reactions require TPNH and diphosphopyridine nucleotide (DPN) respectively It is noteworthy that in contrast to other schemes of intermediary carbohydrate metabolism in which only phosphate esters participate these reactions involve the free sugars Hickman and Ashwell [45] have described a kinase for D xylulose in mammalian liver D Xylulose 5 phosphate will be recognized as an intermediate in the pentose phosphate pathway and may of course be converted to ribose 5-phosphate and to hexose phosphate

tography of deionized urine from several pentosuric subjects Since one ubstance which appeared in all the urines examined by Barnes and Bloomberg had a chromatographic mobility similar to that of xylose some of the author s recent unpublished observations seem pertinent Following the procedure of Barnes and Bloomberg the author too found a xylose like component in the urine of two pentosuric subjects This material was eluted from paper chromatograms and was found by Ashwell [73] to behave like L xylose in several enzymatic reactions However this substance was not pre ent in pentosuric urine subjected to paper chromatography *without* prior exposure to ion exchange resins In addition normal urine to which only L-xylulose was added and which was then deionized and chromatographed on paper also exhibited the xylose spot Accordingly the author has concluded that the appearance of L-xylose in chromatograms of pentosuric urine is an artifact attributable to the preparative procedure

*Site of the Defect*

Although Enklewitz and Lasker [16] demonstrated more than twenty years ago that glucuronic acid administration greatly enhanced the excretion of L-xylulose it was not until the recent elucidation of the glucuronic acid oxidation pathway that a plausible hypothesis could be advanced concerning the biochemical abnormality in pentosuria In our attempts to define the metabolic lesion it will be helpful to cite existing evidence which supports the following propositions

1 Glucuronic acid is a direct precursor of the urinary pentose

2 Glucuronic acid is incompletely metabolized in pentosuric individuals

3 Although the pentosuric person can convert glucuronic acid to L-xylulose a block exists in the further metabolism of the pentose

Touster and his associates [40] confirmed the observation of Enklewitz and Lasker that the ingestion of glucuronolactone is followed by an increased excretion of L-xylulose by pentosuric persons and they postulated that the conversion of glucuronolactone to xylulose is direct They substantiated this hypothe is by two studies in a pentosuric subject [41] First they demonstrated that the administration of glucuronolactone labeled with $C^{14}$ in its sixth carbon atom (the carboxyl carbon) was followed by the excretion of nonisotopic L-xylulose Second they showed that the ingestion of glucuronolactone 1 $C^{14}$ was followed by the urinary excretion of heavily labeled pentose the i otope of which was predominantly in the fifth carbon atom The e data indicate that the carboxyl carbon of glucuronic acid is lost in the conversion to L-xylulose but that the re t of the molecule is pre erved int ict Touster also demon trated that ome normal human beings and guinea pigs excrete traces of L-xylulo e and that this excretion is slightly augmented following glucurono-

ascorbic acid cannot be synthesized Thus it is possible that in man the glucuronic acid oxidation pathway functions only to return a portion of glucuronic acid carbon to the mainstream of carbohydrate metabolism

*Effects of Drugs*

The basis for the effect of drugs and hormones on the glucuronic acid oxidation pathway remains a fascinating but largely unexplored area Margolis's early observation that aminopyrine stimulates the excretion of L-xylulose by individuals with pentosuria [38] was followed by reports indicating that this drug and others such as Chloretone and barbital stimulate free glucuronic acid [68] and ascorbic acid [69] excretion in animals The drug effect is apparently not related to any known detoxification mechanism since barbital is excreted unchanged Hormonal involvement in this process is indicated by the observation of Burns and his associates that the administration of Chloretone or barbital to hypophysectomized rats is not followed by an increase in ascorbic acid excretion [68] It has also been observed that thyroid hormone stimulates pentose excretion in the rat [70] and that the increase in pentose excretion which results from exposure to low temperatures is prevented by the administration of thyroid blocking agents [71] An increase in L-xylulose excretion by normal human beings given triiodothyronine has been reported [72]

## THE BIOCHEMICAL ABNORMALITY IN ESSENTIAL PENTOSURIA

*Nature of the Urinary Sugar*

As has been pointed out there is now conclusive evidence that the urinary sugar excreted by pentosuric persons is L-xylulose (L-xyloketose L-threopentulose) In the light of present knowledge one must conclude that the early purported demonstrations of urinary arabinose either were in error because of deficiencies in the experimental methods employed or else were concerned with patients who do not fit into this category L-Xylulose is excreted in fairly constant amount ranging between 1 0 and 3 5 gm per 24 hr The excretion is increased following the intake of glucuronic acid and of certain drugs but it is unaffected by diet and is not altered by insulin administration

Much smaller quantities of other sugars in addition to L-xylulose have been reported in pentosuric urine Touster and his associates [36] found arabitol and suggested that this sugar alcohol may be derived from L-xylulose reduction Wolf on Cohn and Devaney [37] observed a second unidentified component following chromatography of pentosuric urine and Barnes and Bloomberg [35] found small amounts of several substances in addition to large quantities of L-xylulose following chroma

first 12 hr, 57 per cent of the administered radioactivity appeared in the urine of the normal individual and 76 per cent in that of the pentosuric person Thus the total $C^{14}$ excretion in urine and expired air of the normal and pentosuric subjects during the intervals measured was in excess of 73 and 79 per cent respectively

*Further Isotope Experiments*

The foregoing study strongly supports the view that glucuronic acid metabolism is impaired in pentosuria Some information concerning the site of the defect may be deduced from the results of a published experiment [74] which will be briefly summarized Using a "ribose-trapping technique suggested by the observations of Tabor and Hayaishi [60] and of Karjala [61] that imidazoleacetic acid is excreted in the urine in part as a riboside the author has demonstrated ribose synthesis from hexose in man by way of the oxidative and nonoxidative reactions of the pentose phosphate pathway [53] If the glucuronic acid oxidation pathway were operative in normal individuals it would afford a mechanism for the conversion of glucuronolactone carbon to ribose in the following fashion glucuronolactone ⟶ L-xylulose ⟶ D-xylulose ⟶ D-xylulose 5-phosphate ⟶ D ribose 5 phosphate (For an outline of the reactions involved see Fig 4 2) Thus the administration of imidazoleacetic acid and of glucuronolactone uniformly labeled with $C^{14}$ should be followed by the excretion of imidazoleacetic acid riboside containing ribose-$C^{14}$ On the other hand if this pathway were blocked in pentosuria at a site beyond L-xylulose then a pentosuric subject might be expected to excrete radioactive L-xylulose but nonisotopic riboside ribose (Since there is no reason to postulate an impairment in the pentose phosphate pathway in pentosuria the synthesis and excretion of the riboside ribose should proceed normally) The results of such an experiment are described in Table 4 2 The significant $C^{14}$ incorporation in the urinary riboside ribose of the normal subject is consistent with the conversion of glucuronolactone to ribose by way of the reactions of the glucuronic acid oxidation and the pentose phosphate pathways (Fig 4 2) The pentosuric subject excreted ribose in an amount comparable to that excreted by the normal individual but the virtual absence of radioactivity indicates that it was derived from sources other than glucuronolactone The large quantity of isotope in the urinary L-xylulose is in agreement with Touster s demonstration that glucuronic acid is a direct precursor of the pentose This experiment not only provides information concerning the impairment in pentosuria but also helps to establish the concept that the glucuronic acid oxidation pathway is operative in normal individuals

It must be emphasized that while these experiments provide strong circumstantial evidence that an abnormality in the glucuronic acid

lactone ingestion These observations were considered to strengthen the concept that L-xylulose is a metabolic intermediate in normal subjects

A recent unpublished study may be cited in support of the hypothesis that a defect in glucuronic acid metabolism exists in pentosuria Let us postulate that a pentosuric person can remove carbon atom 6 of glucuronolactone with unimpaired efficiency but that he is incapable of metabolizing the remainder of the carbon chain Then following the administration of glucuronolactone uniformly labeled with $C^{14}$ there should be six

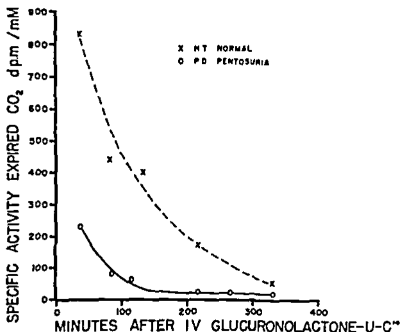



Fig 4-3 $C^{14}O_2$ excretion in the expired air following the intravenous administration of glucuronolactone uniformly labeled with $C^{14}$ to a normal subject and to a pentosuric individual

times as much $C^{14}O_2$ in the expired air of a normal person as is present in that of a pentosuric person These expectations were fulfilled in a study, the results of which are depicted in Fig 4 3 In this experiment 5 microcuries of glucuronolactone uniformly labeled with $C^{14}$ (obtained through the *generosity of Dr N Artz Corn Products Refining Co Argo Ill*) was given intravenously to a 16 year old male with essential pentosuria and to a 22 year old female with no abnormality of carbohydrate metabolism Samples of expired air were collected at intervals for 6 hr thereafter and were analyzed for $CO_2$ and for radioactivity In this period of time 16 per cent of the administered $C^{14}$ appeared in the expired air of the normal subject and 2 6 per cent in that of the pentosuric subject almost exactly the sixfold difference that was predicted In the

two normal individuals a sugar which was identified as xylulose by paper chromatographic techniques (Flynn [79] had previously demonstrated xylulose in pentosuric plasma by chromatography ) After glucurono lactone administration the serum xylulo e level in a pentosuric subject of Bozian and Touster reached 11 mg per 100 ml Simultaneous with the Bozian and Touster study Freedberg Feingold and Hiatt [80] u ed the specific enzymatic assay of Hickman and Ashwell [81] to measure L-xyl ulo e levels in the serum and urine of several non Jewish controls three pentosuric persons and four clo e relatives of pentosuric persons (Table 4 3) From the latter work several points merit emphasis Fasting serum L-xylulo e levels in all three pentosuric persons were in excess of 1 mg per 100 ml while in the erums of all but one of the other individuals no L-xylulose was detectable in the fasting state In all but two subjects D glucuronolactone led to a rise in serum L-xylulo e levels with a peak generally reached within 1 hr of feeding The rise in serum pentose level was accompanied by a marked increase in urinary pentose in the pento suric persons and by the appearance of urinary pentose in all other subjects tested The serum levels of four heterozygotes (two D and E in Table 4 3 are mothers of pentosuric persons a third F is a brother of three per ons with pentosuria G is the child of a pentosuric mother) were higher than those of the normal ubjects and the urine levels in the two heterozygous subjects tested were considerably greater than those in the control subjects As uming normal glomerular filtration rates for the e subjects one can calculate that virtually all L-xylulose entering the renal glomeruli appears in the urine of normal as well as of heterozygous and pentosuric subjects Thus a defect in renal tubular reabsorption of L-xylulose appears to exist in *all* subjects but it is clearly not the metabolic error which distingui hes the pento uric from the normal person

## CLINICAL CONSIDERATIONS

*Manifestations*

Pentosuria can be classified with those inborn errors of metaboli m in which no disturbance of function has been demon trated to re ult from the genetic abnormality Indeed the mo t frequent difficulty en countered by pentosuric subjects is consequent to a mi taken diagno is of diabetes mellitus and the institution thereafter of dietary and insulin therapy A number of reports in the literature indicate that only after an episode of in ulin induced hypoglycemia h is the correct diagnosis been made No abnormality of gluco e metaboli m i demon trable except in those rare in tances in which pento uria and diabetes mellitus coincide Several authors have commented on the frequency and severity of psychologic di turbances encountered in ubjects with pento uria

oxidation pathway exists in pentosuria the enzymatic defect has yet to be demonstrated. Since pentosuria is a benign as well as an uncommon disturbance and further since the enzymes involved in L-xylulose metabolism have thus far been found only in mammalian liver, opportunities to define the abnormal site with precision will be exceedingly sparse. Until such a demonstration conclusions must be tentative.

Knox pointed out that impaired L-xylulose reabsorption by the renal tubule could be the defect present in pentosuria [22]. An experiment which was cited in support of this possibility is that of Enklewitz and Lasker [14] who gave 5 gm L-xylulose by mouth to a pentosuric subject

TABLE 4 ? URINARY L-XYLULOSE AND RIBOSE IN NORMAL AND PENTOSURIC SUBJECTS GIVEN IMIDAZOLEACETIC ACID (ImAA) AND D-GLUCURONOLACTONE UNIFORMLY LABELED WITH $C^{14}$

| | Normal person | Pentosuric subject |
|---|---|---|
| D-glucuronolactone administered counts/min | 1 5 × 10 | 7 8 × 10⁴ |
| μmoles | ?70 | 18. |
| ImAA hydrochloride administered μmoles | .000 | 1 000 |
| Urinary L-xylulose (0 10 hr) μmoles | 0 . | 39.0 |
| Relative molar activity counts/min/μmole | | 85 |
| Total C content counts/min | 300 | 3 30 × 10⁴ |
| Per cent administered $C^{14}$ | 0 00 | 4 3 |
| Ribose from urinary ImAA riboside (0 10 hr) | | |
| μmoles | ?? | 08 |
| Per cent administered ImAA | 14 | 21 |
| Relative molar activity counts/min/μmole | 33 | |
| Total $C^{14}$ content counts/min/μmole | 9 100 | 100 |
| Per cent administered $C^{14}$ | 0 0.8 | 0 00. |

Significance doubtful because of small quantity of radioactivity

and found only 0 5 gm additional L-xylulose in the urine. This suggested metabolism of the pentose. However in the absence of information concerning the efficiency of L-xylulose absorption from the human gastrointestinal tract the Enklewitz and Lasker experiment must be interpreted with caution. Indeed there are published studies which indicate that although parenteral administration of L-xylulose to animals is followed by extensive metabolism [40 75 76] the pentose orally administered is not glycogenic in rats [77]. Moreover even if considerable absorption takes place the possibility exists that the absorbed pentose might inhibit endogeneous L-xylulose production by way of a feed back mechanism.

Following the publication of Knox's review two studies appeared which convincingly exclude the renal abnormality hypothesis. Bozian and Touster [78] found in the plasma of a pentosuric subject but not of

but a causal relationship between such disturbances and the error in carbohydrate metabolism has not been established and the suggestion is often made that at least some neurotic complaints may be related to the conflicting medical opinions to which many patients have been subjected Lasker has followed 40 pentosuric individuals for periods in excess of 16 years and has found no decrease in life expectancy as compared with normal individuals [20] Some typical clinical considerations may best be presented by citing a hitherto unpublished report of a case of pentosuria first diagnosed in 1958

P D a 16 year-old high school sophomore was first seen in the Out Patient Department of the Beth Israel Hospital in February 1958 One year previously a physician had found a reducing substance in the patient s urine and had placed him on a low carbohydrate diet Despite faithful adherence to this regimen the urine continued consistently to show a 1+ reaction in the Benedict s test For this reason his physician suggested instituting insulin therapy and the patient s father brought him to the Beth Israel Hospital to seek additional opinion The patient had never noted polydipsia polyuria or polyphagia and during the 6 months prior to admission he had gained 8 lb in weight The father could not recall a single negative urine sugar test during this period but at no time did he find more than a 1+ reduction There was a family history of diabetes mellitus in a paternal great uncle but not of other known disturbances of carbohydrate metabolism The patient s parents were Austrian born Jews who were not consanguineous and whose forebears had come from Poland His past medical history was not contributory except in two important respects Ten years and six years previously the patient had been seen in the Pediatric Clinic of the Beth Israel Hospital for upper respiratory infections Urinalyses carried out on both occasions were recorded as having shown a 1+ positive Benedict s test In addition he had frequently been seen by psychiatrists during the previous decade because of problems of behavior During the year prior to admission his emotional disturbances were apparently magnified by his concern over his condition and also by his resentment at the dietary restrictions to which he had been subjected

Physical examination revealed a well-developed well nourished young male who appeared in good health Vital signs were normal and no significant abnormalities were found on examination Laboratory studies revealed a normal hemogram a 2 hr postprandial blood sugar of 95 and a urinalysis that was normal except for a 1+ positive test for a reducing substance The urine however did not give a positive reaction with Testape (an enzyme impregnated paper which reacts specifically with glucose) The urine sugar was shown to be L-xylulose by preparation of the osazone and by paper chromatography in n butanol-ethanol water (50 10 40) with authentic L-xylulose as a standard followed by staining with the orcinol trichloroacetic acid reagent [8 ] A glucose tolerance test (50 gm glucose by mouth) showed a fasting blood sugar of 80 mg per 100 ml and blood glucose levels at 30 60 120 180 and 240 min of 120 110 95 80 and 60 mg per 100 ml respectively The patient s 24 hr urinary excretion of L-xylulose was found to be 2 gm and this rose to 4 gm during the 24 hr following the ingestion of 5 gm of D-glucuronolactone The patient and his parents were reassured concerning the benign nature of this disturbance in carbohydrate metabo-

Table 4-3 Serum and Urine L-Xylulose Levels before and after Oral Administration of D-Glucuronolactone

| Subject | Amount of glucuronolactone gm | Serum L-Xylulose | | | Urine L-Xylulose | | |
|---|---|---|---|---|---|---|---|
| | | Fasting mg/100 ml | Maximal mg/100 ml | Time after glucuronolactone min | Fasting mg/hr | Maximal mg/hr | Time after glucuronolactone hr |
| Pentosuric subjects | | | | | | | |
| A | 5 | 1 [illegible] | [illegible] | 60 | | 3 0 | 1-2 |
| B | 10 | 1 3 | 5 [illegible] | 60 | 106 | 541 | 1-2 |
| C | 10 | 1 [illegible] | 14 [illegible] | 30 | 88 | 450 | 1- |
| Pentosuric relatives | | | | | | | |
| D | 5 | | 1 6 | 60 | | | |
| E | 5 | | 3 9 | 30 | | | |
| F | 25 | 0 18 | 0 41 | 30 | | 81 | 0 1 |
| G | 25 | | 1 41 | 30 | 0 1 | 63 | 0 1 |
| Control subjects | | | | | | | |
| H | 25 | | | | | 9 | 0-1 |
| I | 5 | | | | | 22 | 0-1 |
| J | 5 | | 0 2[illegible] | 90 | | 16 | 0-1 |
| K | 25 | | 0 29 | 90 | 0 6 | 13 | 0 1 |
| L | 25 | | 0 15 | 60 | 0 3 | 18 | 1-2 |

Less than 0 1 mg per 100 ml

has a characteristic mobility ($R_F = 0.26$) which exceeds those of other commonly observed urinary sugars [82] and gives a red color on staining with the orcinol trichloroacetic acid reagent [83]

3 *Cysteine-carbazole test* [84]

4 *Behavior of Osazone* [29] The phenylosazone of L-xylulose has a melting point of about 160 C When it is mixed with the osazone of D-xylose the crystalline appearance is radically altered (Fig 4-4) and the melting point rises approximately 40

## GENETIC CONSIDERATIONS

Estimates of the incidence of pentosuria vary the most widely accepted figure 1 in 50 000 is derived from studies of applicants for life insurance examinations [77] The age at which the diagnosis has first been made varies from 2 to 61 years but it is likely that pentosuria is present from birth The earlier reports that pentosuria occurs predominantly in males [12] is not substantiated by studies of the families of pentosuric persons where the incidence seems evenly divided between the sexes The thesis that pentosuria behaves as a simple recessive anomaly was first proposed by Garrod [12] and has been amply confirmed Lasker Enklewitz and Lasker [18] found 37 instances of pentosuria in 20 families They set forth the following evidence to support their view that pentosuria behaves as a recessive characteristic

1 Despite its rarity in the general population they found 10 new cases among 34 brothers and sisters of pentosuric propositi

2 Most cases are among Jews (A recent report demonstrating with chromatographic techniques pentosuria in two sisters of Lebanese descent [35] is the first instance of proved xylulosuria in non Jews )

3 The presence of the abnormality in the children of 10 families in which neither parent had pentosuria indicates that the condition is recessive In the 10 sibships 16 of 38 members had pentosuria Deducting the 8 propositi leaves 8 of 30 members with pentosuria a figure consonant with the Mendelian ratio for a recessive characteristic when both parents are heterozygous

4 One of their subjects was a child of first cousins (one of the parents had pentosuria)

Reference has already been made to studies of serum and urinary L-xylulose [80] which are now continuing in an effort to obtain more information concerning the transmission of the pentosuria gene Thus far a defect in L-xylulose metabolism after (but *not* before) glucuronolactone administration has been shown in two mothers of individuals with pentosuria in a man who is one of five siblings three of whom have been shown to have pentosuria and in the son of one of the latter siblings with pentosuria

lism and specifically concerning the absence of any relationship of his condition to diabetes mellitus He was returned to an unrestricted diet and according to his parents within a month many of the behavioral disturbances which had been present during the previous year were greatly diminished

*Diagnostic Measures*

A diagnosis of pentosuria should be suspected in any person and particularly in a Jewish person, who has none of the symptoms of diabetes mellitus but in whose urine a small quantity of a reducing substance is consistently found This possibility is strengthened in those instances in which the urine does not give a positive test with any of the enzymatic methods specific for glucose The measures which have proved most




Fig 4-4 Osazone crystals of L-xylulose (A) D-xylose (B) and a mixture of A and B (C) (*By permission of H D Barnes et al [35]*)

useful in establishing the diagnosis of pentosuria may be summarized as follows

1 *Reduction of Benedict s reagent at low temperature [15]* L-Xylulose is a strong reducing substance and in contrast to glucose and most other urinary sugars will reduce Benedict s reagent at 55 C in 10 min or at room temperature in 3 hr (Fructose will also reduce Benedict s reagent at low temperature )

2 *Paper chromatography* On paper chromatography L-xylulose can be readily distinguished from other sugars For example with a mixture of *n* butanol ethanol and water (50 10 40) as the solvent L-xylulose

13 Klercker K O Studien uber die Pentosurie Nor l me l ark 38 1 1 05
14 Enklewitz M and Lasker M Studies in pentosuria A report of l cases Am J M Sc 186 539 1933
15 Lasker M and Enklewitz M A simple method for the detection and e timation of L-xyloketo e in urine J Biol Chem 101 289 1933
16 Enklewitz M and Lasker M The origin of L-xyloketo e (urine pento e) J Biol Chem 110 443 1935
17 Enklewitz M and Lasker M Pento uria in twins J A M A 105 950 1935
18 Lasker M Enklewitz M and La ker G W The inheritance of L-xyloketosuria (essential pento uria) Human Biol 8 43 1936
19 Lasker M The question of arabinosuria Am J Clin Path 20 485 1950
20 Lasker M Mortality of persons with xyloketosuria Human Biol 27 J4 1955
21 Derivaux R C Essential pentosuria (xyloketosuria) South M J 36 587 1943
22 Knox E Sir Archibald Garrod s inborn errors of metabolism IV Pentosuria Am J Human Genet 10 385 1958
23 Neuberg C Ueber die Harnpentose ein optisch inactives Naturlich vorkommendes Kohlehydrat Ber deutsch chem Gesell ch 33 ? 43 1900
24 Cammidge P J and Howard H A N S ven ca es of e sential pentosuria Brit M J 2 ?? 19 0
25 Aron H I nfall von Pentosuria im fruhen Kindesalter Monatsschr Kinderh 12 177 1913
26 Luzzatto R Ricerches dans un cas de pento urie chronique Arch ital biol 51 469 1909
27 Schuller L Ueber inaktive und rechtsdrehende Arabino e aus el eidung im Harn Berl klin Wchnschr 47 13 ? 1910
28 Zerner F and Waltuch R Ein Beitrag zur Kenntnis der Pentosuri vom chemischen Standpunkt Monatsschr Chem 34 16 9 1913
29 Levene P A and LaForge F B Note on a case of pentosuria J Biol Chem 15 319 1914
30 Zerner E and Waltuch R Zur Frage des Pentosuriezuckers Biochem Ztschr 58 410 1914
31 Hiller A The identification of the pentose in a case of pentosuria J Biol Chem 30 1 9 1917
32 Greenwald I The nature of the sugar in four cases of pentosuria J Biol Chem 88 1 1930
33 Solis Cohen S Essential pentosuria Am J M Sc 139 349 1910
34 Solis Cohen S and Gershenfield L Supplemental report of a case of essential pentosuria of twenty-eight years standing Am J M S 192 610 1936
35 Barnes H D and Bloomberg B M Paper chromatography of the urinary sugar in essential pentosuria South African J M Sc 18 93 1953
36 Touster O and Harwell S The isolation of L-arabitol from pentosuri urine J Biol Chem 230 1031 19 8
37 Wolfson W G Cohn C and Devaney W A An improved apparatus and procedure for ascending chromatography on large size filter p per sheets Science 109 541 1949
38 Margolis J I Chronic pentosuria and migraine Am J M Sc 177 348 19 9
39 Everett M R *Med cal Biochemistry* p 31 Hoeber New York 194
40 Touster O Hutcheson R M and Rice L The influence of D-glucuronolactone on the excretion of L-xylulose by humans and guinea pigs J Biol Chem 215 677 1955
41 Touster O Mayberry R H and McCormick D B The conversion of 1 C-D-glucuronolactone to 5- C-L-xylulose in a pento uric human Biochem et biophys acta 25 196 1957
42 Touster O Reynolds V H and Hutcheson R M The reduction of L-xylulose to xylitol by guinea pig liver mitochondria J Biol Chem 221 697 1956
43 Hollman S and Touster O The L-xylulose xylitol enzyme and other polyol dehydrogenases of guinea pig liver mitochondria J Biol Chem 225 8 1957

## SUMMARY

1 Essential pentosuria is an inborn error of metabolism in which 1 0 to 4 gm of the pentose L-xylulose is excreted in the urine each day It is a benign disturbance which occurs almost solely in Jews and which behaves genetically as an autosomal recessive characteristic

2 This disorder bears no relationship to diabetes mellitus and is easily distinguished from several other varieties of pentosuria in which milligram quantities of a number of pentoses other than L-xylulose appear in the urine

3 Essential pentosuria is almost surely the result of a defect in the glucuronic acid oxidation pathway In this route of carbohydrate metabolism the carboxyl carbon atom of D glucuronic acid is removed in a series of reactions giving rise to the pentose L-xylulose The latter may then be converted to its stereoisomer D-xylulose which in turn may be phosphorylated D Xylulose 5 phosphate may participate in reactions of the pentose phosphate pathway which lead to its conversion to hexose phosphate (Glucuronic acid ⟶ gulonic acid ⟶ L-xylulose ⟶ xylitol ⟶ D xylulose ⟶ pentose phosphate pathway ⟶ hexose phosphate ) The glucuronic acid oxidation pathway apparently serves no essential function in man

4 Strong evidence has been cited to indicate a block in this pathway at some reaction beyond the L-xylulose step in individuals with pentosuria

## BIBLIOGRAPHY

1 Salkowski E and Jastrowitz M Ueber eine bisher nicht beobachtete Zuckerart im Harn *Centr med Wissensch* 30 337 1892
2 Garrod A E The lessons of rare maladies Lancet 1 1055 1928
3 *The Works of William Harvey M D* translated by Robert Willis p 616 Sydenham London 1847 (Cited in ref 2 )
4 Peters J P and Van Slyke D D *Quantitative Clinical Chemistry Interpretations* vol I Williams & Wilkins Baltimore 1946
5 Hawk P B Oser B L and Summerson W H *Practical Physiological Chemistry* pp 844–845 McGraw Hill-Blakiston New York 1954
6 Johnstone R W Pentosuria chronic and alimentary Edinburgh M J 20 138 1906
7 Tower D B Peters E L and Pogorelskin M A Nature and significance of pentosuria in neuromuscular disease Neurology 6 37 [illegible] 1956
8 Touster O Hutcheson R M and Reynolds V H The formation of L-xylulose in mammals and its utilization by liver preparations J Am Chem Soc 76 5005 1954
9 Futterman S and Roe J H The identification of ribulose and L-xylulose in human and rat urine J Biol Chem 215 257 1955
10 Kiliani H Ueber die Zusammensetzung und Constitution der Arabinosecarbonsaure bezw der Arabinose Ber deutsch chem Gesellsch 20 339 1887
11 Janeway T C Essential pentosuria in two brothers Am J M Sc 132 423 1906
12 Garrod A E Inborn errors of metabolism Lancet 2 217 1908

71 Roe J H and Coover M O Effect of cold adrenocorticotropic and thyroid hormones on urinary excretion of pentose in the rat Proc Soc Exper Biol & Med 74 146 1950
72 Baker E M Plough I C and Berman E L Alternate pathways of glucose metabolism in man factors influencing the excretion of ketopentose Clin Res Proc 6 406 1958
73 Ashwell G Personal communication
74 Hiatt H H Studies of ribose metabolism IV The metabolism of D-glucurono lactone in normal and pentosuric subjects Biochem et biophys acta 28 645 1958
75 Greenwald I The possible significance of L-xyloketose (urine pentose) in normal metabolism J Biol Chem 91 731 1931
76 Larson H W Chambers W H Blatherwick N R Ewing M E and Sawyer S D The metabolism of D- and L-xylulose in the depancreatized dog J Biol Chem 129 701 1939
77 Larson H W Blatherwick N R Bradshaw P J Ewing M E and Sawyer S D The metabolism of L-xylulose J Biol Chem 138 353 1941
78 Bozian R C and Touster O Essential pentosuria renal or enzymic disorder Nature London 184 463 1959
79 Flynn F V Essential pentosuria Brit M J 1 391 1955
80 Freedberg I Feingold D and Hiatt H H Serum and urine L-xylulose levels in normal and pentosuric individuals and in subjects with pentosuria trait Biochem Biophys Res Communications 1 328 1959
81 Hickman J and Ashwell G A sensitive and stereospecific enzymatic assay for xylulose J Biol Chem 234 758 1959
82 Lederer E and Lederer M *Chromatography* Elsevier Amsterdam 1955
83 Hough L Jones J K N and Wadman W H Quantitative analysis of mixtures of sugars by the method of partition chromatography Part V Improved methods for the separation and detection of the sugars and their methylated derivatives on the paper chromatogram J Chem Soc 170 1950
84 Dische Z and Borenfreund E A new spectrophotometric method for the detection and determination of keto sugars and trioses J Biol Chem 192 583 1951

44 Ashwell G Enzymatic degradation of D-galacturonic and D-glucuronic acid Fed Proc 16 146 1957
45 Hickman J and Ashwell G Purification and properties of D-xylulokinase in liver J Biol Chem 232 737 1958
46 Ashwell G, Kanfer J and Burns J J Studies of the mechanism of L-xylulose formation by kidney enzymes J Biol Chem 234 47 1959
47 Burns J J and Kanfer J Formation of L-xylulose from L-gulonolactone in rat kidney J Am Chem Soc 79 3604 1957
48 ul Hassan M and Lehninger A L Enzymatic formation of ascorbic acid in rat liver extracts J Biol Chem 223 123 1956
49 Horecker B L and Hiatt H H Pathways of carbohydrate metabolism in normal and neoplastic cells New England J Med 258 177 225 1958
50 Horecker B L and Mehler A H Carbohydrate metabolism Ann Rev Biochem 24 207 1955
51 Marks P A and Feigelson P Biosynthesis of nucleic acid ribose and of glycogen glucose in the rat J Biol Chem 226 1001 1957
52 Hiatt H H Studies of ribose metabolism II A method for the study of ribose synthesis in vivo J Biol Chem 229 725 1957
53 Hiatt H H Studies of ribose metabolism VI Pathways of ribose synthesis in man J Clin Invest 37, 1461 1958
54 Horecker B L and Smyrniotis P Z The coenzyme function of thiamine pyrophosphate in pentose phosphate metabolism J Am Chem Soc 75 1009 1953
55 Hiatt H H Studies of ribose metabolism V Factors influencing in vivo ribose synthesis in the rat J Clin Invest 37 1453 1958
56 Horecker B L Gibbs M Klenow H and Smyrniotis P Z The mechanism of pentose phosphate conversion to hexose monophosphate I With a liver enzyme preparation J Biol Chem 207 393 1954
57 Hiatt H H Studies of ribose metabolism III The pathway of ribose carbon conversion to glucose in man J Clin Invest 37, 651 1958
58 Hiatt H H Studies of ribose metabolism I The pathway of nucleic acid ribose synthesis in a human carcinoma cell in tissue culture J Clin Invest 36 1408 1957
59 Perkoff G T and Tyler F H Studies in disorders of muscle XI The problem of pentosuria in progressive muscular dystrophy Metabolism 5 563 1956
60 Tabor H and Hayaishi O The excretion of imidazoleacetic acid riboside following the administration of imidazoleacetic acid or histamine to rats J Am Chem Soc 77 505 1955
61 Karjala S A The partial characterization of a histamine metabolite from rat and mouse urine J Am Chem Soc 77 504 1955
62 Hammarsten E Reichard P and Saluste E Pyrimidine nucleosides as precursors of pyrimidines in polynucleotides J Biol Chem 183 105 1950
63 Mosbach E H and King C G Tracer studies of glucuronic acid biosynthesis J Biol Chem 185 491 1950
64 Eisenberg F Jr and Gurin S The biosynthesis of glucuronic acid from 1 C14-glucose J Biol Chem 195 317 1952
65 Ginsberg V Weissbach A and Maxwell E S Formation of glucuronic acid from uridinediphosphate glucuronic acid Biochim et biophys acta 28 649 1958
66 Hiatt H H Studies of ribose metabolism VII An assessment of ribose biosynthesis from hexose by way of the C-6 oxidation pathway J Biol Chem 233 1023 1958
67 Isherwood F A Chen Y T and Mapson L W Synthesis of L-ascorbic acid in plants and animals Biochem J 56 1 1954
68 Burns J J Evans C Trousof N and Kaplan J Stimulatory effect of drugs on excretion of L-ascorbic acid and non-conjugated D glucuronic acid Fed Proc 16 286 1957
69 Longenecker H E Fricke H H and King C G The effect of organic compounds upon vitamin C synthesis in the rat J Biol Chem 135 497 1940
70 Roe J H and Coover M O Role of the thyroid gland in urinary pentose excretion in the rat Proc Soc Exper Biol & Med 75 818 1950

## METABOLISM OF FRUCTOSE

The keto-hexose fructose is widely distributed among plants and is an important source of dietary carbohydrate It is present as the free mono saccharide or in polysaccharides such as sucrose and less frequently as inulin and raffinose The open chain form of the hexose is in equilibrium with the corresponding pyranose and furanose forms In sucrose the fructose in glycosidic linkage is in the furanose form whereas in solutions of the free sugar the pyranose form predominates

### Intestinal Absorption

Fructose polysaccharides are split in the intestine to yield free fructose as the end product of digestion Fructose as such is not actively transported by the intestinal mucosa as are the other common hexoses glucose and galactose [9] Some of the fructose is converted to glucose in the intestinal mucosa [10 35] The remainder that is not further metabolized crosses the mucosa by the process of passive diffusion The fraction of fructose which is converted to glucose in the passage across the intestinal wall varies considerably from species to species For example considerably greater conversion occurs in the guinea pig than in the rat [11] In man it has been estimated that about one sixth of the fructose absorbed is converted to glucose by the intestinal mucosa [12] This occurs by way of the fructose 1 phosphate pathway discussed in detail below

### Plasma Turnover

Once absorbed fructose is rapidly metabolized In normal man the half time of disappearance of fructose from the blood following intravenous injection is about 18 min The concentration of fructose in the blood in the postabsorptive state is normally low (2 6 ± 1 9 mg per 100 ml) [13] Following an oral fructose load of 40 to 50 gm in normal individual the fructose concentration in the blood rises only slightly in 30 to 60 min and rarely above 10 to 20 mg per 100 ml (fructose tolerance test) [3 4] Less than 2 to 3 per cent of the orally administered fructose appears in the urine

### Biochemical Sequences of Fructose Metabolism

Knowledge of the pathways of fructose metabolism has been obtained from many species Consequently the following discussion is based upon experiments in different animals Considerable variation exists from tissue to tissue and the exact role of each remains to be determined The known reactions are summarized in Fig 5-1 The numbers used in this discussion refer to this figure

# Chapter 5

# Fructosuria

*Stephen M Krane*

## DEFINITION

Fructosuria is a rare disorder of metabolism in which there are defective utilization and excessive excretion in the urine of the common hexose fructose With the exception of the cases described by Froesch et al [*1 36*] and that reported by Chambers and Pratt [*2*] the condition is harmless and asymptomatic Instances of fructosuria are usually discovered in the course of routine testing of urine for reducing substance which upon further investigation proves to be fructose

The criteria for the diagnosis of fructosuria may be simply stated [*3–5*]

1 The sugar must be identified as fructose i e it is fermentable by yeast gives a positive Seliwanoff reaction and is levorotatory Its identity should be confirmed by chromatography [*6*] The specimen tested must be freshly voided since fructose may be formed from glucose in alkaline urine in the presence of some bacteria [*7*]

2 The subject must have normal liver function since fructose may be excreted in amounts greater than normal in hepatic failure [*7*]

3 Usually fructose in the urine is dependent upon its intake in the diet when the sugar is eliminated from food the fructosuria diminishes

4 The standard glucose tolerance test result is normal

5 The condition is not influenced by insulin

Fructosuria appears in both sexes in approximately equal incidence It has been reported in all age groups from birth to 89 years [*7*] The condition is extremely rare Marble reported only 4 cases among 29 000 cases of mellituria seen at the Joslin Clinic [*8*] and Lasker estimated that the incidence in the general population is approximately one in 130 000 [*7*]

In order to consider the metabolic defect in fructosuria it is first necessary to discuss the normal metabolism of fructose

Fructose 1 phosphate can be phosphorylated to fructose 1 6 diphosphate in the presence of 1 phosphofructokinase (*Reaction 4*) This enzyme is present in muscle but not in liver [16] No direct isomerase for the conversion of fructose 1 phosphate to glucose 1 phosphate has been demonstrated [16]

**Reactions 5 and 6** Fructose 1 phosphate can be split by an aldolase to dihydroxyacetone phosphate and glyceraldehyde [20] Studies of Leuthardt suggest that fructose 1 phosphate aldolase may be a different enzyme from fructose 1 6 diphosphate aldolase [21] Hers on the other hand has suggested that a single aldolase catalyzes both *Reactions 5 and 6* but with different substrate affinities (the $K_m$ for fructose 1 phosphate is 100 times that for fructose 1,6 diphosphate) [20]

**Reactions 7 to 10** The glyceraldehyde formed by splitting fructose 1 phosphate is phosphorylated in the presence of ATP by a triosekinase to glyceraldehyde 3 phosphate (*Reaction 7*) [20] This can then condense with dihydroxyacetone phosphate in the presence of aldolase to form hexose diphosphate [20] In liver and muscle another enzyme has been identified (*Reaction 8*) fructose 1 6 diphosphatase which in the presence of magnesium ion splits the phosphate specifically from carbon 1 of fructose 1 6 diphosphate to yield fructose 6 phosphate and inorganic phosphate [20] The fructose 6-phosphate can be converted to glucose 6 phosphate (*Reaction 9*) in the presence of phosphohexoseisomerase and with glucose 6-phosphatase (*Reaction 10*) in liver free glucose may be formed [16 20] Thus a series of reactions has been demonstrated for the conversion of fructose to glucose in the liver

There is another series of reactions for the interconversion of fructose and glucose In the seminal vesicles glucose is reduced to sorbitol by TPNH The sorbitol in turn is oxidized to fructose by DPN These reactions account for the high concentration of fructose in the semen [22 23]

*Utilization of Fructose*

In man 40 to 50 per cent of the fructose is removed by the liver and planchnic tissues during a constant infusion [12 13 24] Other estimates of total metabolism of fructose by the liver are as high as 80 per cent [3] Fructose freely enters hepatic cells [25] The mechanism of utilization of fructose in the presence of glucose by hepatic cells has been outlined above There is still considerable uncertainty concerning the extent of metabolism of fructose by muscle Although utilization of fructose has been demonstrated in isolated muscle and by measurement of peripheral arteriovenous differences in the presence of glucose this is small compared to glucose utilization [24] In the isolated diaphragm conversion of fructose to glycogen when equal concentrations of glucose and fructose are present is only one-sixth that of glucose and conversion to carbon dioxide only one-third that of glucose [24] In the eviscerated nephrec

**Reaction 1** Fructose can be phosphorylated at position 6 in the presence of ATP magnesium ion and hexokinase The enzyme which has been most carefully studied is that located in the particulate fractions of brain It catalyzes the phosphorylation of fructose but the affinity of the enzyme for fructose is considerably lower than for glucose [14] Therefore at equimolar concentrations of glucose and fructose phosphorylation of fructose by this enzyme is completely inhibited [15] If the relative affinities of other tissue hexokinases are similar to that of brain hexokinase then this enzyme has little importance for phosphorylation to fructose 6-phosphate because of the unfavorable ratios of glucose to fructose

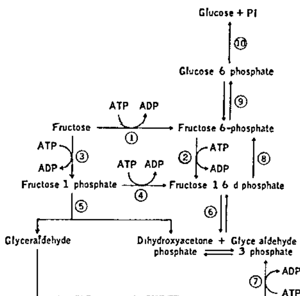



Fig 5-1 The pathways of fructose metabolism The reaction numbers refer to the text

**Reaction 2** Fructose 6 phosphate is further phosphorylated to fructose 1 6 diphosphate in the presence of magnesium ions ATP and phosphofructokinase The activity of this system is low in liver but high in muscle [16] Under conditions of high fructose concentration muscle phosphofructokinase can phosphorylate fructose in the 1 position because of its lack of specificity [17] However there is probably little physiologic significance to the latter reaction

**Reaction 3** In liver and possibly also in muscle another enzyme fructokinase catalyzes the phosphorylation of fructose in the 1 position has an absolute requirement for potassium and magnesium ions and is inhibited noncompetitively by ADP [16 18 19] In contrast to the less specific hexokinase glucose is not a substrate for fructokinase and does not competitively inhibit the phosphorylation of fructose by the latter enzyme

Sachs Sternfeld and Kraus reviewed 55 cases of fructosuria reported until 1942 and added 2 cases of their own [4] Several cases have been published since then [3 8 30 31] The following discussion represents a summary of the material in these reports

*Abnormalities in Fructose Utilization*

In the majority of patients no more than traces of fructose are found in the urine and blood in the fasting state An exception is the patient reported by Lenzner who had a fasting blood fructose of 41 mg per 100 ml and 0 4 gm fructose in a 2 hr urine collection after 15 hr of fasting [31] However following an oral fructose load or the ingestion of fructose either as the mono- or polysaccharide the hexose appears in the urine Within 3 to 4 hr following fructose ingestion 10 to 20 per cent of the sugar appears in the urine in contrast to normal subjects who excrete only 1 to 2 per cent under similar circumstances On an average diet the fructosuric subject rarely excretes urine with fructose concentrations greater than 2 to 3 per cent but values fluctuate widely depending upon intake [3 4 8]

The patient with fructosuria shows a significant rise in blood fructose following fructose feeding Whereas normal persons rarely reach concentrations higher than 10 to 20 mg per 100 ml after ingesting 50 gm fructose fructosuric patients often show rises as high as 65 mg per 100 ml 1 to 2 hr later [1] In several fructose tolerance tests in fructosuric subjects the blood glucose has fallen at the time when fructose concentrations have reached peak values [4] When one subject was given 50 gm each of glucose and fructose simultaneously the urinary excretion of fructose was greater than that with fructose alone This was interpreted as indicating that glucose competitively inhibits the utilization of fructose [3]

Sachs Sternfeld and Kraus in their review [4] cited earlier observations that in fructosuria the expected rise in respiratory quotient (R Q ) and in blood lactic acid following fructose ingestion did not occur [32 33] Heeres and Vos observed that their patient with fructosuria had an increase in R Q from 0 72 only to 0 77 following 50 gm fructose orally whereas a normal person treated similarly had a rise in R Q from 0 71 to 0 95 [33] In the two cases of fructosuria reported by Sachs et al the increases in blood lactic acid were 2 and 1 mg per 100 ml 1 hr after fructose ingestion compared with increases of 13 and 12 mg per 100 ml in two controls [4] In their fructosuric persons serum inorganic phosphate did

[1] The levels of blood fructose reported by Lenzner [31] in both the fructosuric and control subjects are higher than any others reported On the basis of the blood values the fructose tolerance test result of his control subject would be considered markedly abnormal and compatible with the diagnosis of fructosuria Although in the fructose method used by him glucose is stated not to interfere details of recovery experiments are not given

tomized rabbit the rate of formation of $C^{14}O_2$ from fructose-$C^{14}$ is only 5 per cent of that from glucose-$C^{14}$ [26]. Recent experiments of Hers using fructose-1-$C^{14}$ have shown that most of the fructose converted to muscle glycogen is derived from the glucose formed from fructose in the liver [27]. The studies of Sols have also suggested that fructose utilization (fructose-1-phosphate formation) by muscle extracts is a side activity of phosphofructokinase [17]. There are few data concerning fructose utilization by other tissues. Erythrocytes, for example, utilize fructose only in the absence of glucose [1]. Lactic acid production by leukocytes in equimolar concentrations of fructose and glucose is one-quarter that with fructose alone [28].

The available evidence thus suggests that the important pathway for fructose utilization is through the liver rather than through muscle, in contrast to glucose. Infusions of fructose to normal man produce a considerably greater rise in lactic and pyruvic acids and a greater fall of inorganic phosphate in the serum than similar infusions of glucose [13]. The hepatic uptake and conversion of fructose to lactate, carbon dioxide and glycogen exceeds that of glucose [24]. However, studies of Smith, Ettinger, and Seligson indicate that the rates of utilization of infused fructose and glucose are approximately equal [13]. These authors therefore have suggested that the differences in the ability of the two hexoses to produce a rise in lactate and pyruvate is accounted for by the entrance of fructose into the Embden-Meyerhof scheme at a different level than glucose. The first product of fructose metabolism, fructose-1-phosphate, is immediately metabolized to 3-carbon fragments, as was discussed above.

The renal threshold for fructose is apparently quite low; urine to plasma ratios of fructose of less than 1.0 are found only at plasma concentrations below 15 mg per 100 ml [29]. At fructose levels higher than this, some sugar will appear in the urine. Smith has summarized the evidence which suggests active reabsorption of fructose by the renal tubules from the glomerular filtrate [29].

Several studies have shown that fructose utilization is normal in diabetic man [12, 13]. The rate of fructose disappearance following intravenous infusion is only slightly prolonged in diabetic human subjects and changes in blood lactic, pyruvic, $\alpha$-ketoglutaric acids, and inorganic phosphate are of the same magnitude as in normal persons [13]. Insulin fails to influence fructose utilization [3, 13].

## THE METABOLIC ABNORMALITY IN BENIGN FRUCTOSURIA

In the light of the previous discussion of fructose metabolism, the abnormalities in fructosuria may be considered together with hypotheses regarding the mechanism of the defect.

The child gradually learned to avoid sweets Aside from slight enlargement of the liver small stature and decreased intelligence no other abnormalities were found Glucose and galactose tolerance test results were normal There was no hypersensitivity to fructose by skin testing *Paper chromatography* of the urine revealed some increase in amino acids The child was given 50 gm fructose per square meter of body surface and fructose and glucose (the latter by glucose oxidase method) were determined in blood and urine Within 40 min the child became pale cyanotic irritable and began to sweat excessively The blood pressure rose from 115/65 before the test to 135/85 in 4 hr At 40 min the blood glucose had fallen from a fasting concentration of 65 mg per 100 ml to 35 mg per 100 ml and the blood fructose had risen from trace amounts to over 80 mg per 100 ml Ninety minutes after the test was begun the blood fructose reached a peak of 138 mg per 100 ml and the blood glucose was 8 mg per 100 ml After 4 hr there was still profound hypoglucosemia and hyperfructosemia The blood glucose did not begin to rise until after 6 hr, when the blood fructose had fallen to below 30 mg per 100 ml and slight clinical improvement began The patient was still nauseated and exhausted on the following day Two days after the test mild icterus and albuminuria developed During the study only 10 per cent of the fructose was excreted in the urine

A similar clinical picture was observed in the 2½ year old brother of the patient reported above Mention is also made of two adult relatives of these children who were strong and healthy in appearance but who had avoided fruits and sugar because during early life these had produced episodes of nausea vomiting and somnolence A smaller fructose load was given to one of these The blood fructose rose from a fasting level of 54 mg per 100 ml to a peak of 86 mg per 100 ml The blood glucose dropped concomitantly to 20 mg per 100 ml and the drop was accompanied by hunger sweating and palpitations Fructose tolerance test results in the parents of the two children described above were normal Peak values of fructose in the blood were only 17 mg per 100 ml and only traces of fructose appeared in the urine during the test Of six parents of the four patients described four could be traced to common ancestors in the eighteenth century

The authors explain the abnormality in these patients as follows They note that in previous studies of fructosuria mild decreases in blood glucose concentrations have been seen in the course of fructose tolerance tests Although it had been observed that in cross-circulation experiments in dogs [34] there was no stimulation of insulin production with fructose the authors suggest that this does occur in man In normal man the conversion of fructose to glucose in the liver is prompt and serves to correct the hypoglucosemia to some extent In their patients this fructose-to-glucose conversion is blocked

not fall as it normally does following fructose ingestion. A similar failure to demonstrate a rise in lactic acid in fructosuria was noted by Levine and Lahenhaus [3].

It can be seen that there is considerable evidence that the utilization of fructose is decreased in fructosuric subjects. In these individuals following the ingestion of fructose there is an excessive rise in blood fructose and a failure both in production of lactic acid and $CO_2$ (failure of R.Q. to increase) and in consumption of inorganic phosphate. In addition there is a decrease in the conversion of fructose to glucose as may be inferred from the results of fructose tolerance tests in fructosuric subjects: there is a slight fall in glucose concentration in the blood in contrast to a slight rise in normal persons. It has often been stressed that recovery of fructose in the urine of fructosuric subjects is rarely greater than 20 per cent of the load. It is then assumed that 80 per cent of the sugar is "utilized" [3]. "Disappearance" can be equated with "utilization" only when products of metabolism can be demonstrated, as has been pointed out by Smith et al. [13]. In fructosuria no increase in fructose metabolites (such as lactate) occurs after fructose ingestion. What happens to the remaining 80 per cent of the fructose load is unknown.

*Suggested Enzymatic Defects*

At the present time the evidence favors the interpretation that the defect in fructosuria is a deficiency of hepatic fructokinase (Reaction 3) [1]. An alternative explanation places the defect not at fructokinase but in the utilization of fructose-1-phosphate (fructose-1-phosphate-aldolase, Reaction 5). As mentioned previously, the enzyme for the conversion of fructose-1-phosphate to fructose-1,6-diphosphate is not present in the liver. A defect in the utilization of fructose-1-phosphate in liver could account for the disappearance of fructose not excreted in the urine and not appearing as lactate, glucose, or $CO_2$. Fructose-1-phosphate would then be expected to accumulate in the cells. It must be emphasized that the evidence is inferential and indirect and will probably remain so until there are opportunities for directly measuring the activities of the enzymes involved in fructose metabolism in affected persons.

## FRUCTOSURIA WITH HYPOGLUCOSEMIA

Although usually no clinical disturbance is associated with fructosuria, a recent report by Froesch et al. documents striking fructose intolerance in several members of a Swiss family [1]. Their first patient was a 6½ year old girl who was retarded in growth and intellectual development. The child's mother had recognized since age 8 months that frequent attacks of vomiting, sweating, somnolence and tremor were associated with the ingestion of cane sugar and fruits. Pure glucose was well tolerated

2 The defect is not found to occur in any of the parents or offspring of the affected subjects

3 In 6 of 7 families where the facts are known the parents of the patients are related in some manner

Froesch et al conclude that the inheritance in their cases of fructosuria with hypoglucosemia is an autosomal recessive [1] The same criteria proposed by Lasker would be fulfilled in this instance i e occurrence in siblings nonoccurrence in parents and evidence of consanguinity in the parents of the affected subjects

## TREATMENT

No treatment is indicated in the benign form of fructosuria since there are no symptoms and no evidence that there are any deleterious effects from fructose ingestion The only clinical hazard is confusion of the condition with diabetes mellitus The development of specific and simple tests for urine glucose involving glucose oxidase has minimized this danger [2] For those subjects with fructosuria and hypoglucosemia absolute restriction of fructose in all forms in the diet is mandatory if cerebral damage is to be avoided

## SUMMARY

1 Fructosuria is usually a benign asymptomatic metabolic anomaly Following ingestion of fructose affected persons do not metabolize the sugar normally There results a high concentration of fructose in the blood and failure of the blood lactate or respiratory quotient to rise in contrast to what is observed in normal persons

2 There is no evidence to suggest a renal defect but the sugar is excreted in the urine when the renal threshold is surpassed Although definitive studies have not been reported it is possible that a deficiency in fructokinase of liver and other tissues is responsible for the defect

3 Another group of patients has been described who have profound depression in glucose concentration in the blood associated with hyperfructosemia and fructosuria following the ingestion of fructose It has been postulated that in these subjects the defect in fructose utilization involves a deficiency of fructose 1 phosphate aldolase or glyceraldehyde kinase

4 Both forms of fructosuria are probably inherited as Mendelian recessive trait

[2] On the other hand if tests using glucose oxidase for sugar in the urine are widely adopted for routine screening purposes and less specific tests for reducing substances are discarded then interesting abnormalities such as fructosuria will go undetected

In a more recent report Froesch et al [36] have made several additional observations on these interesting subjects They found that when a *small* dose of fructose (3 gm per m² of body surface) was given intravenously to the younger brother of the patient mentioned above a fast exaggerated and prolonged fall in serum inorganic phosphorus occurred in contrast to what occurred in a normal slightly older subject On the basis of this observation they postulated that fructose was normally phosphorylated to fructose 1 phosphate and that this ester accumulated within the cell because of a primary deficiency of fructose 1 phosphate aldolase Unfortunately the authors were unable to obtain liver biopsies to prove this and their conclusions are therefore inferential In one experiment with a rat liver homogenate the authors observed 60 to 70 per cent inhibition of fructokinase with fructose 1 phosphate present at a concentration of $5 \times 10^{-3}$ M Inhibition of fructokinase by fructose 1 phosphate was not previously noted [16] Using crystalline aldolase they observed competitive inhibition of this enzyme by fructose 1 phosphate and calculated an inhibition constant to be about $10^{-3}$ M ($K_i$) On the basis of these observations they postulated that accumulation of fructose 1 phosphate could inhibit fructose utilization as well as glucose formation via the aldolase reaction

In attempting to explain the difference between their cases and the usual patients with benign fructosuria Froesch et al have speculated that in their cases the defect is in fructose 1 phosphate aldolase (Reaction 5) or glyceraldehyde kinase (Reaction 7) They make the valid observation that it is improbable that their cases are homozygous and those with benign fructosuria are heterozygous for the same defective gene since the parents of their cases had normal fructose tolerance test results Whether or not this is a qualitatively different form of fructosuria will be decided only by definitive localization of the defect

Chambers and Pratt have reported the case of a 24 year-old woman who had recurrent vomiting following ingestion of fruit or cane sugar [5] The peak blood fructose level was 17 mg per cent at 60 min after eating 50 gm fructose No blood glucose data were reported

## GENETICS

The most careful study of the mode of inheritance of fructosuria is that of Lasker [7] She reviewed the literature and her own case and concluded that the disorder is inherited as a Mendelian recessive trait Her arguments are as follows

1 Of the 19 families on whom adequate data are available 10 show the defect in more than one sibling In 15 families where there is information on the total size of the sibship 40 brothers and sisters of the original cases were tested for fructosuria and 7 were found to be positive

2 The defect is not found to occur in any of the parents or offspring of the affected subjects

3 In 6 of 7 families where the facts are known the parents of the patients are related in some manner

Froesch et al conclude that the inheritance in their cases of fructosuria with hypoglucosemia is an autosomal recessive [1] The same criteria proposed by Lasker would be fulfilled in this instance i e occurrence in siblings nonoccurrence in parents and evidence of consanguinity in the parents of the affected subjects

## TREATMENT

No treatment is indicated in the benign form of fructosuria since there are no symptoms and no evidence that there are any deleterious effects from fructose ingestion The only clinical hazard is confusion of the condition with diabetes mellitus The development of specific and simple tests for urine glucose involving glucose oxidase has minimized this danger [2] For those subjects with fructosuria and hypoglucosemia absolute restriction of fructose in all forms in the diet is mandatory if cerebral damage is to be avoided

## SUMMARY

1 Fructosuria is usually a benign asymptomatic metabolic anomaly Following ingestion of fructose affected persons do not metabolize the sugar normally There result a high concentration of fructose in the blood and failure of the blood lactate or respiratory quotient to rise in contrast to what is observed in normal persons

2 There is no evidence to suggest a renal defect but the sugar is excreted in the urine when the renal threshold is surpassed Although definitive studies have not been reported it is possible that a deficiency in fructokinase of liver and other tissues is responsible for the defect

3 Another group of patients has been described who have profound depression in glucose concentration in the blood associated with hyperfructosemia and fructosuria following the ingestion of fructose It has been postulated that in these subjects the defect in fructose utilization involves a deficiency of fructose 1 phosphate-aldolase or glyceraldehyde kinase

4 Both forms of fructosuria are probably inherited as Mendelian recessive traits

[2] On the other hand if tests using glucose oxidase for sugar in the urine are widely adopted for routine screening purposes and less specific tests for reducing substances are discarded then interesting abnormalities such as fructosuria will go undetected

In a more recent report Froesch et al [36] have made several additional observations on these interesting subjects They found that when a *small* dose of fructose (3 gm per m² of body surface) was given intravenously to the younger brother of the patient mentioned above a fast exaggerated and prolonged fall in serum inorganic phosphorus occurred in contrast to what occurred in a normal slightly older subject On the basis of this observation they postulated that fructose was normally phosphorylated to fructose 1 phosphate and that this ester accumulated within the cell because of a primary deficiency of fructose 1 phosphate aldolase Unfortunately the authors were unable to obtain liver biopsies to prove this and their conclusions are therefore inferential In one experiment with a rat liver homogenate the authors observed 60 to 70 per cent inhibition of fructokinase with fructose 1 phosphate present at a concentration of $5 \times 10^{-3}$ M Inhibition of fructokinase by fructose 1 phosphate was not previously noted [16] Using crystalline aldolase they observed competitive inhibition of this enzyme by fructose 1 phosphate and calculated an inhibition constant to be about $10^{-3}$ M (K) On the basis of these observations they postulated that accumulation of fructose 1 phosphate could inhibit fructose utilization as well as glucose formation via the aldolase reaction

In attempting to explain the difference between their cases and the usual patients with benign fructosuria Froesch et al have speculated that in their cases the defect is in fructose 1 phosphate aldolase (Reaction 5) or glyceraldehyde kinase (Reaction 7) They make the valid observation that it is improbable that their cases are homozygous and those with benign fructosuria are heterozygous for the same defective gene since the parents of their cases had normal fructose tolerance test results Whether or not this is a qualitatively different form of fructosuria will be decided only by definitive localization of the defect

Chambers and Pratt have reported the case of a 24 year old woman who had recurrent vomiting following ingestion of fruit or cane sugar [2] The peak blood fructose level was 17 mg per cent at 60 min after eating 50 gm fructose No blood glucose data were reported

## GENETICS

The most careful study of the mode of inheritance of fructosuria is that of Lasker [7] She reviewed the literature and her own cases and concluded that the disorder is inherited as a Mendelian recessive trait Her arguments are as follows

1 Of the 19 families on whom adequate data are available 10 show the defect in more than one sibling In 15 families where there is information on the total size of the sibship 40 brothers and sisters of the original cases were tested for fructosuria and 7 were found to be positive

2 The defect is not found to occur in any of the parents or offspring of the affected subjects

3 In 6 of 7 families where the facts are known, the parents of the patients are related in some manner

Froesch et al conclude that the inheritance in their cases of fructosuria with hypoglycosemia is an autosomal recessive [1] The same criteria proposed by Lasker would be fulfilled in this instance i e occurrence in siblings nonoccurrence in parents and evidence of consanguinity in the parents of the affected subjects

## TREATMENT

No treatment is indicated in the benign form of fructosuria since there are no symptoms and no evidence that there are any deleterious effects from fructose ingestion The only clinical hazard is confusion of the condition with diabetes mellitus The development of specific and simple tests for urine glucose involving glucose oxidase has minimized this danger [2] For those subjects with fructosuria and hypoglycosemia absolute restriction of fructose in all forms in the diet is mandatory if cerebral damage is to be avoided

## SUMMARY

1 Fructosuria is usually a benign asymptomatic metabolic anomaly Following ingestion of fructose affected persons do not metabolize the sugar normally There result a high concentration of fructose in the blood and failure of the blood lactate or respiratory quotient to rise in contrast to what is observed in normal persons

2 There is no evidence to suggest a renal defect but the sugar is excreted in the urine when the renal threshold is surpassed Although definitive studies have not been reported it is possible that a deficiency in fructokinase of liver and other tissues is responsible for the defect

3 Another group of patients has been described who have profound depression in glucose concentration in the blood associated with hyperfructosemia and fructosuria following the ingestion of fructose It has been postulated that in these subjects the defect in fructose utilization involves a deficiency of fructose 1 phosphate aldolase or glyceraldehyde kinase

4 Both forms of fructosuria are probably inherited as Mendelian recessive traits

[2] On the other hand if tests using glucose oxidase for sugar in the urine are widely adopted for routine screening purposes and less specific tests for reducing substances are discarded then interesting abnormalities such as fructosuria will go undetected

## BIBLIOGRAPHY


1 Froesch E R Prader A Labhart A Stuber H W and Wolf H P Die hereditäre Fructoseintoleranz eine bisher nicht bekannte kongenitale Stoffwechselstoring Schweiz med Wchnschr 87 1168 1957

2 Chambers R A and Pratt R T C Idiosyncrasy to fructose Lancet 2 340 1956

3 Levine R and Tabenhaus M Clinical conference on metabolic problems Essential fructosuria Metabolism 2 8 1954

4 Sachs B Sternfeld L and Kraus G Essential fructosuria its pathophysiology Am J Dis Child 63 252 1942

5 Marble A Non-diabetic melituria in The Treatment of Diabetes Mellitus edited by E P Joslin H F Root P White and A Marble Lea & Febiger Philadelphia 1959

6 Cohen A S and Kantor N A simplified paper chromatographic method for separation of glucose and fructose in urine Am J Clin Path 25 1318 1955

7 Lasker M Essential fructosuria Human Biol 13 51 1941

8 Marble A The diagnosis of the less common meliturias including pentosuria and fructosuria M Clin North America 31 313 1947

9 Wilson T H and Vincent T N Absorption of sugars in vitro by the intestine of the golden hamster J Biol Chem 216 851 1955

10 Darlington W A and Quastel J H Absorption of sugars from isolated surviving intestine Arch Biochem 43 194 1953

11 Kiyasu J Y and Chaikoff I L On the manner of transport of absorbed fructose J Biol Chem 224 935 1957

12 Miller M Craig J W Drucker W R and Woodward H Jr The metabolism of fructose in man Yale J Biol & Med 29 335 1956

13 Smith L H Jr Ettinger R H and Seligson D A comparison of the metabolism of fructose and glucose in hepatic disease and diabetes mellitus J Clin Invest 32 273 1953

14 Sols A and Crane R K Substrate specificity of brain hexokinase J Biol Chem 210 581 1954

15 Slein M W Cori G T and Cori C F A comparative study of hexokinase from yeast and animal tissues J Biol Chem 186 763 1950

16 Cori G T Ochoa S Slein M W and Cori C F The metabolism of fructose in liver Isolation of fructose 1 phosphate and inorganic pyrophosphate Biochim et biophys acta 7 304 1951

17 Sols A and Ruffo A Hexophosphorylations Bull soc chim biol 39 Suppl II 71 1957

18 Parks R E Jr Ben Gershon E and Lardy H A Liver fructokinase J Biol Chem 227 231 1957

19 Hers H G La fructokinase du foie Biochim et biophys acta 8 416 1952

20 Hers H G and Kusaka T Le metabolisme du fructose 1 phosphate dans le foie Biochim et biophys acta 11 427 1953

21 Leuthardt F and Wolf H P Fructose 1 phosphate aldolase from liver in Methods in Enzymology edited by S P Colowick and N O Kaplan vol I Academic Press Inc New York 1955

22 Mann T Biochemistry of Semen Wiley New York 1954

23 Hers H G Le mecanisme de la transformation de glucose en fructose par les vésicules seminales Biochim et biophys acta 22 [illegible] 1956

24 Renold A E and Thorn G W Clinical usefulness of fructose Am J Med 19 163 1955

25 Cahill G F Jr Ashmore J Renold A E and Hastings A B Blood glucose and the liver Am J Med 26 264 1959

26 Wick A N Sherrill J W and Drury D R The metabolism of fructose by the extra hepatic tissues Diabetes 2 465 1953

27 Hers H G The conversion of fructose 1 C and sorbitol 1 C to liver and muscle glycogen in the rat J Biol Chem 214 373 1955

28 Martin S I McKinney G R Green R and Becker C The influence of glucose fructose and insulin on the metabolism of leukocytes of healthy and diabetic subjects J Clin Invest 32 1171 1953
29 Smith H W The Kidney Structure and Function in Health and Disease Oxford University Press New York 1951
30 Broman B Essential fructosuria Acta paediat Suppl 75 83 1949
31 Lenzner A R Fructosuria report of a case Ann Int Med 45 70 1956
32 Rynbergen H J Chambers W H and Blatherwick N R Respiratory metabolism in fructosuria J Nutrition 21 553 1941
33 Heeres P A and Vos H Fructosuria Arch Int Med 44 47 1929
34 Lozza G Galansino G Hoffeld H and Foa P P Stimulation of insulin output by monosaccharides and monosaccharide derivatives Am J Physiol 192 497 1958
35 Salomon L L and Johnson J E Transfer of fructose and glucose across surviving guinea pig intestine Arch Biochem Biophys 82 179 1959
36 Froesch E R Prader A Wolf H P and Labhart A Die hereditäre Fructoseintoleranz Helvet paediat acta 14 99 1959

# Chapter 6

# Glycogen Deposition Diseases*

---

*Richard A Field*

Glycogen deposition diseases occupy a noteworthy place in a treatise on the biochemical aspects of hereditary diseases for the demonstration by Gerty T Cori in 1952 [1] that von Gierke's disease is due to the loss of activity of a single known tissue enzyme marked the first clear delineation of an inborn error of metabolism in enzymatic terms and served as a prototype for future work in this group of diseases The contributions of specific enzymologic studies of tissues from patients with genetically determined metabolic aberrations in which Dr Cori led the way, have already been voluminous and significant The demonstration of the practicability of a new conceptual approach to the study of the diseases was among Dr Cori's greatest contributions and will continue to adorn the memory of this great scientist and thinker To those who knew her and had the experience of working with her the memory of her imagination energy and power of intellect will always serve as a source of inspiration [2]

As summarized by Dr Cori in her last publication on the subject [3] enzymatic investigations have made it possible to classify cases of glycogen deposition diseases according to a specific enzyme defect and the resulting alterations of structure of the accumulated tissue glycogen Such a classification is based on an ever-expanding knowledge of the successive steps of glycogen formation and breakdown A review of these facts follows

## METABOLISM OF GLYCOGEN

Glycogen a glucosyl polymer polysaccharide has a role as a reserve material in animal carbohydrate metabolism analogous to the role of

* This work was supported in part by grants from the Public Health Service (2A-507 and A 1435)

starch in plant life Apparently every tissue of the body can form glycogen from glucose glycogen is most abundant in the liver and muscle Under normal conditions in man there are 300 gm of muscle glycogen and 150 to 200 gm of liver glycogen Approximately 25 per cent of the usual 150 to 350 gm of carbohydrate in the diet is stored as glycogen Although this storage polymer of glucose is available in other tissues as a source of hexose phosphate for their intrinsic metabolic needs only the liver can release significant quantities of free glucose into the blood for transport to other tissues In the rat the biologic half life of liver glycogen is 1 day whereas it is 3 to 4 days elsewhere in the body This reflects the primary role of the liver in maintaining normoglycemia and carbohydrate homeostasis The ability of hepatic glycogen to supply glucose to the blood stream is almost entirely dependent upon the presence of a relatively specific hepatic glucose 6 phosphatase An identical or very similar specific glucose 6-phosphatase is found in the kidneys and islet cells of the pancreas [4] (and perhaps in the mucosa of the ileum) but it seems unlikely that these organs contribute much dephosphorylated glucose to the blood stream The presence of this enzyme in the proximal convoluted tubules of the kidney [5] suggests that its role in this organ concerns tubular reabsorption of glucose from the glomerular filtrate (cf Chap 38)

*Structure of Glycogen*

This polysaccharide is a high molecular weight polydisperse polymer (2 5 to 4 5 million average molecular weight) consisting of only one building block α D glucose [6] Many molecules contain more than 10 000 glucosyl units The prevalent link between the glucose residues is in the alpha configuration through C atoms 1 and 4 In addition there are a lesser number of α 1 6 glucosidic bonds which constitute branch points and impart an arborized structure to the molecule The percentage of glucosyl moieties involved in formation of these branch points varies between 6 and 8 per cent of the total in glycogens from different organs or from different species A free reducing group exists only at one terminus of the molecule The uninterrupted succession of 1 4 linked glucosyl components terminates after successive branching and subbranching at 1 6 linkage points with an end devoid of reducing capacity In this ramified structure the external chains which are situated beyond the last 1 6 branch point are much longer than the internal chain located between two points of ramification The outer chains consist of from 7 to 10 glycosyl units in man and constitute approximately 50 per cent of the mass of the molecule while the average length of the inner chains is four glucose residues [7] The resultant structure is more highly branched than amylopectin the analogously branched component of starches and consequently both inner and outer chains of glycogen are shorter This is correlated with a

much higher solubility of glycogen than of amylopectin in water and also accounts for a Bordeaux color reaction with iodine rather than the purplish one obtained with starch. There may be other differences between these two types of polysaccharides, nevertheless it seems justified and useful to regard them as belonging to a family of closely related molecules [3] serving similar physiologic roles (Fig. 6-1).

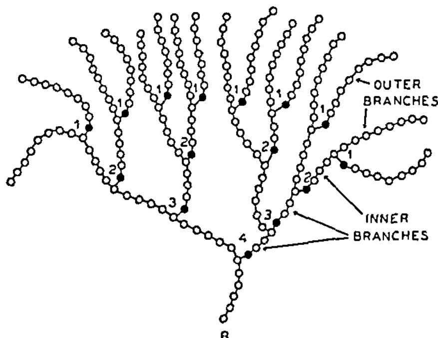



Fig. 6-1. Model of a segment of a glycogen molecule. There are [illegible] glucose residues, molecular weight 33,858. Open circles, glucose residues in α 1-4 linkage; *R* = reducing end group. There are four tiers of branch points (glycogen has at least seven). Inner branches are terminated by branch points in adjacent tier; outer branches by a branch point and by the *nonreducing terminal glucose residue (end group).* *(By permission of C. T. Cori [3].)*

*Synthesis and Degradation of Glycogen*

In the various classified types of abnormal glycogen deposition syndromes the lesions *involving the glycogen molecule are both quantitative and qualitative. Normally liver glycogen content in man varies* from 0 to 5 per cent, depending on circumstances. Muscle glycogen is only rarely as high as 1 per cent of the wet weight of the tissue. Excessive deposition of glycogen to the extent of 15 per cent of wet weight of liver (approximately 50 per cent of the dry weight) and up to 12 per cent of the wet weight in the muscle has been found [8]. The classification and characterization of the qualitative disturbances *of the structure* of the glycogen molecule are best described as logical consequences of specific

Glucosyl (1 4) + glucosyl (1 6)

Amylo (1 4 → 1 6) t n gl cos d e (b anch )

(Gl cosyl (1 4)) +1 + UDP

Glycogen

UDPG glycogen tra sgluc yl se

Gluc syl (1 4)

UDP Glucose + PP

UTP

UDPG py ophosphorylase

Phosphoryl se

Glucose 1 phosphate

Phosphogl comutase

Glucose —He ok na e→ Glucose 6 phosphate

Ph sphohe ose Isomerase

P ATP ADP

(O idat ve phosphoryl t )

Fructose 6 phosphate

(Gl coneogenesis)

A

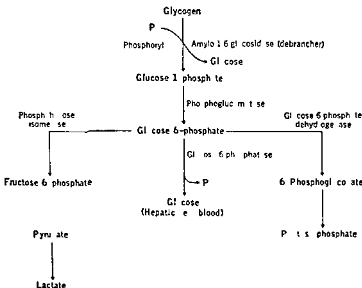



Fig 6- A Synthesis of glycogen B Degradation of glycogen

enzyme defects in pathways traversed by glucose in the synthesis of glycogen and in the breakdown of glycogen to yield again free glucose. These reactions bear close scrutiny inasmuch as they influence the availability of glycogen and are central in carbohydrate metabolism. Disorders of glycogen synthesis and limitations of the availability of glucose from glycogen can understandably exert far reaching effects demonstrable chiefly as hypoglycemia, impairment of synthetic processes and of emergency release of energy and limitation of growth. The schema of Fig 6-2A and B above summarizes current knowledge of these steps in the liver (Fig 6-2)

Presentation of glycogenesis and glycogenolysis as separate pathways enhances the clarity of the discussion of the individual enzymes. The recent demonstration of UDPG[1] glycogen transglucosylase by Leloir [9] has provided a mechanism for a division of the two processes beyond the stage of glucose 1 phosphate formation. As pointed out by Robbins et al [10] and Leloir et al [11] in their more elaborate characterization of this enzyme, such a partition would resolve the long standing paradox between the classical concept of reversible interconversion of glycogen and glucose 1 phosphate and the findings of Sutherland and coworkers [12-14] and of Cori [15] that activation of phosphorylase in vivo invariably leads to glycogen breakdown and never to glycogen synthesis. It is tempting to add as further evidence for partition the demonstrated ability of human muscle lacking phosphorylase activity to synthesize glycogen. As yet direct evidence excluding phosphorylase from participation in glycogen synthesis in the intact normal animal has not been presented. Nevertheless it seems likely that such evidence will be forthcoming. Thus it has been stressed by Stetten and others that the normal tissue concentrations of inorganic phosphate are relatively high in relation to determined concentrations of glucose 1 phosphate. This relationship would keep the phosphorylase reaction well over on the breakdown side of its equilibrium (see below)

### GLYCOGENOLYTIC ENZYMES

*Phosphorylase*

This enzyme in vitro is specific for the phosphorylytic breaking of the prevalent $\alpha$ 1-4 linkage in glycogen (Fig 6-3)

In vitro the reaction is easily reversible with an equilibrium constant [16]

$$\frac{\text{(Phosphate)}}{\text{(Glucose 1 phosphate)}} = 2.45 \text{ at pH } 7.33$$

Rabbit muscle phosphorylase as crystallized by Green and Cori [17] and called phosphorylase *a* has more than 60 per cent of its activity without

[1] Uridine diphosphoglucose

addition of nucleotides but requires the addition of adenosine 5 monophosphate for maximal activity in vitro Phosphorylase *a* is apparently a tetramer which contains 4 moles of pyridoxal phosphate per mole [18] It can be converted by the hydrolytic PR or phosphorylase rupturing enzyme abundantly present in muscle tissue into a dimer with

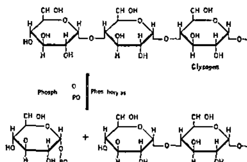



Fig 6-3 The active phosphorylase reaction in vitro (*By permission of C T Cori* [3]

an absolute requirement for AMP for activity [19] The inactivating cleavage reaction has been summarized as [20]



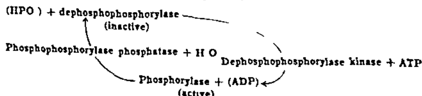
$$\text{Phosphorylase } a + 4\text{ H O} \longrightarrow 2\text{ phosphorylase } b + 4\text{ HPO}$$

Recondensation of two dimers into an active *a* tetramer requires a specific phosphorylase *b* kinase and ATP and proceeds as follows [21]



$$2\text{ Phosphorylase } b + 4\text{ ATP} \longrightarrow \text{phosphorylase } a + 4\text{ ADP}$$

Liver possesses a phosphorylase which is immunologically distinct and biochemically different from the phosphorylase of muscle [22] but which also exists in an active and inactive form In the case of hepatic phosphorylase in contrast to the situation with muscle phosphorylase deactivation of the active form by a specific enzyme does not involve a cleavage The active and inactive forms have the same molecular weight Sutherland and Wosilait [23] purified the active form and Sutherland and coworkers [24] described a system of phosphorylation and dephosphorylation of hepatic phosphorylase in which the amount of active phosphorylated enzyme present at any instant is the resultant of the continuous dynamic actions of the activating and deactivating enzyme The situation can be described graphically as follows



The activity of the phosphorylated enzyme can be increased 15 to 40 per cent in vitro by the addition of AMP Glycogenolytic activity of liver tissue at any given instant seems to be directly proportional to its content of active phosphorylated phosphorylase [25–27] which is in keeping with the demonstration that the phosphorylase step is rate limiting in glycogenolysis [28] The relative amount of active phosphorylase, as will be discussed in detail later can be greatly enhanced by the actions of epinephrine and glucagon invariably with a concomitant increase in breakdown of glycogen This is a further indication of the nonintervention of phosphorylase in glycogenesis and supports the notion that synthesis occurs primarily via UDPG and the Leloir enzyme A deficiency of muscle phosphorylase also to be described later results in a form of excessive glycogen deposition in muscles, a circumstance which would be hard to understand if the enzyme were required in both formation and depolymerization of the polysaccharide Thus pending direct evidence on the point it seems proper to assign to phosphorylase as its chief if not sole physiologic role the phosphorolysis of glycogen and to postulate that synthesis occurs chiefly via the Leloir enzyme If phosphorylase does participate in synthesis to any degree then some more subtle circumstances of physical partition within the cells or more complicated influence for intermittent reversal of the direction of action than the phosphate glucose 1 phosphate ratio must exist

*Amylo-1 6-glucosidase*

In its action during glycogenolysis phosphorylase successively attacks the most distal $\alpha$ 1 4 glucosidic bonds of the outer branches of the treelike glycogen molecule In the presence of adequate concentrations of inorganic phosphate the successive phosphorolytic cleavages continue until the outermost tiers of $\alpha$ 1 6 glucosidically bound glucosyl moieties are approached Since crystalline or purified phosphorylase cannot attack this linkage action stops and the results are an accumulation of glucose 1-phosphate stoichiometric consumption of inorganic phosphate and a partially depolymerized glycogen tree exhaustively pruned of its outer branches The partially depolymerized but not yet deramified polysaccharide which results from exhaustive phosphorylase action has been termed the first limit dextrin (phosphorylase) or $LD_1$ (phosphorylase) (Fig 6-4)

$LD_1$ (phosphorylase) is not identical with the one produced by the plant enzyme $\beta$ amylase The latter can approach the branch points more closely while splitting off the disaccharide maltose The $LD_1$ ($\beta$ amylase) is a smaller molecule than $LD_1$ (phosphorylase) as can be appreciated by study of Fig 6-4

Another enzyme amylo-1 6 glucosidase present in muscle and presumably liver catalyzes the hydrolytic cleavage of the 1 6 linked glucose

residues [29] This enzyme appropriately called debrancher acts only on the LD1 LD2 LD3 etc (phosphorylase) in which the glucosyl residues in 1 6 linkage are no longer covered by additional glucose residues It has no significant activity on undigested glycogen amylopectin or on LD1 (β-amyla e) The debrancher reaction is from physiologic considera tions irreversible In splitting the branch point with the introduction of $H_2O$ it yields free glucose rather than glucose 1 phosphate

Limit dextrin (phosphorylase) ⟶ deramified glycogen + glucose ı

Although only 8 per cent of the glycogen appears as free gluco e via this mechanism when glycogen is enzymatically degraded in vitro the

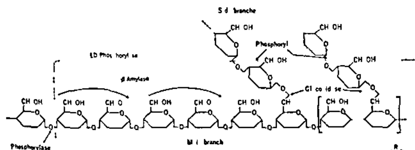



Fig 6-4 Structural model of portion of a branched poly accharide showing tl e sites of enzymatic action *LD* corresponds to the limit dextrin formed by exhaustive action of pl osphorylase *I* refers to the reducing end (*By permission of G T Cori [3]*)

recognition of this potential source of free glucose is important in under standing the occurrence of hyperglycemia following administration of glucagon and epinephrine in certain of the glycogen deposition diseases

The purification of this enzyme and its removal as a contaminant in the preparation of pho phorylase by repeated crystallizations of the latter have provided investigational tools for determination of the struc ture of various glycogens and an elegant method of biochemical diag nosis [30] By the successive actions of crystalline phosphoryla e and purified debrancher in a tierwi e fa hion on purified glycogen the length of the outer chains number of branch points in any given tier length of inner chains and number of tiers can be deduced As will be seen this method *of characterization of qualitatively abnormal glycogens led to* the postulation of pecific enzyme le ion Eventually direct a say of the enzyme in question proved that the hypothe is based on enzymatic structure analy is was correct [31] Thu by the joint action of phos phorylase and amylo-1 6 gluco idase a molecule of glycogen can be almo t completely degraded to gluco e 1 pho phate and gluco e leaving as a re idue an oligo accharide whose molecular weight and tructure

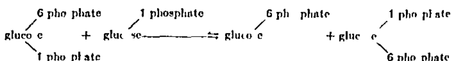
are not well known



$$\text{Glycogen} + n\,HPO_4 + m\,H_2O \longrightarrow n\ \text{glucose 1 phosphate} + m\ \text{glucose} + \text{oligosaccharide}$$

*Phosphoglucomutase*

Abnormalities of this enzyme have not been described as a cause of biochemical pathologic change Much information has been obtained concerning its mode of action It has been surmised that absence or deficiency of this enzyme would be lethal [8] because of its bottleneck position in glycogen metabolism The enzyme mediates the interconversion of glucose 1 phosphate and glucose 6-phosphate and requires glucose 1 6-diphosphate as an obligate cofactor in vitro [32] The reaction is thought to proceed as follows




At equilibrium the mixture contains 95 per cent glucose 6-phosphate and 5 per cent glucose 1 phosphate [33], which makes it difficult to see how glycogen synthesis is affected via this enzyme if the initial source material is glucose 6 phosphate nevertheless no specific evidence is available which casts doubt on its intervention in the formation of glycogen as well as in its degradation Interference with phosphoglucomutase activity by galactose 1 phosphate has been demonstrated [34 35] and postulated as an important cause for the problems seen in galactosemic children fed on galactose or lactose (Chap 7)

*Glucose 6 phosphatase*

This microsomal enzyme accomplishes the hydrolytic cleavage of phosphate from glucose 6-phosphate liberating free glucose and inorganic phosphate [36 37] It is normally present in abundance only in the liver and kidneys [38] a circumstance which explains the anatomic distribution of excessive glycogen deposition in these organs when lesions of this enzyme exist Liver has other phosphatases which are capable of attacking glucose 6 phosphate but under physiologic conditions the specific glucose 6 phosphatase dephosphorylates at least ten times the amount of glucose 6 phosphate as the nonspecific enzymes [8] The nonspecific predominantly acid phosphatases of liver have very little activity in the physiologic range of pH 6 2 to 6 8 in which the optimum for the specific microsomal enzyme lies [1] Further glucose 6-phosphatase has a preferential affinity for glucose 6-phosphate (Michaelis constant $5 \times 10^{-3}$ M) [39] and there is little splitting of other hexose 6-phosphates when they are tested with this enzyme in the same concentration [40]

The enzyme attacks pho phate esters of primary alcohols with con siderable avidity [39]

It is essential to the understanding of the manifestations and responses of subjects bearing a deficiency of this enzyme to recognize its influential and critical position in the regulation of the blood sugar In the fasting state glucose 6 phosphate derived either from glycogenolysis or from the formation of glucose from other than carbohydrate sources (gluconeogenesis) requires the intercession of this enzyme before it can leave the liver and enter the blood as free glucose With the exception of the small amount of glucose formed during deramification by amylo-1 6-glucosidase all blood sugar of endogenous origin comes via glucose 6-phosphatase

An interesting and probably physiologically important property of this enzyme is that its level of assayable activity is decidedly influenced by hormones drugs and circumstances which are attended by shifts in the direction of liver glucose metabolism [41] The assayable activity rises in diabetes starvation or whenever the liver is provoked to increase its output of free glucose Conver ely circumstances such as hyperalimenta tion or administration of insulin or sulfonylurea [42] are attended by a drop in activity of this enzyme These changes in enzyme activity occur only in vivo and require some time and persistent change in conditions to become manifest [43] Increases and decreases in activity seem to parallel the presumed changes in intracellular glucose 6-phosphate con centration or production rising in response to the persistence of increased levels of the substrate and falling following sustained declines in the availability of the substrate It is not clear whether these adaptive changes are brought about by increa ed enzyme synthe is such as have been described for so-called adaptive enzymes or whether shifts in levels of activity repre ent alterations in a ratio of active and inactive forms of a relatively fixed number of enzyme molecules such as seems to be the case with liver phosphorylase Treatment of microsomal par ticles rich in glucose 6-pho phata e activity with Triton and other detergents has been shown to increase markedly the activity in vitro [42] suggesting a possible mechanism by which hifts between an active and an altered active form might be effected in vivo It is conceivable that the clinical examples of deficiencies of this enzyme activity al though they are almost absolute represent a genetically determined defect in an activating-deactivating system rather than an ab olute inability to produce the enzyme molecule The question is more than academic since the po sibility of a therapeutic approach to the deficient activity syndrome might be easier if the problem is to find a method of activating an existing enzyme rather than to induce *de novo* production of enzyme molecules or to induce a metabolic by pass Sub titution therapy by supplying glucose 6-pho phatase from exogenous sources seems hardly possible on immunologic grounds and the improbability

of the enzymes finding its proper place inside the proper cell has been pointed out by Cori [3]

## GLYCOGENIC ENZYMES

Only a single instance of a disorder of glycogen synthesis marked by excessive glycogen deposition and an abnormally structured polysaccharide has come under study [31] Despite this apparent paucity of examples of disordered glycogen synthesis recent discoveries concerning this obviously important side of the glycogen cycle make it profitable to discuss the steps involved in some detail

### *Hexokinase*

The concept that phosphorylation occurs during entry of sugars into cells has been under assault in recent years as a result of studies on the mechanisms of insulin action [45] and intestinal absorption [46] The liver cell has long been recognized as relatively highly permeable to free glucose and it is not difficult to demonstrate definite concentrations of this hexose in the intracellular water of hepatic cells [47] In order to enter the steps of glycogen formation or carbohydrate metabolism glucose and other sugars require phosphorylation by ATP and a "kinase The properties of muscle hexokinase [48] and of brain hexokinase [49] which mediate the transfer of the terminal phosphate from ATP to C6 of glucose have been extensively studied The liver is more difficult to study because of the apparent lability of the enzymes interferences by ATPases and the difficulties in ridding preparations of phosphoglucomutase and other contaminating enzymes A specific glucokinase for liver which phosphorylates in the 6 position has been postulated [50] For fructose and galactose [50 51] specific kinases mediate phosphorylation of the hexoses in the 1 position


Galactose + ATP ⟶ galactose 1 phosphate + ADP

Through a series of reactions discussed in detail in the chapter on galactosemia (Chap 7) galactose 1 phosphate can furnish glucose 1 phosphate and thus glucosyl units for addition to the ends of the outer chains of the glycogen molecule without the intercession of phosphoglucomutase Supporting this concept [52] are the incorporation studies with galactose 2 $C^{14}$ and glucose 2 $C^{14}$ which show less randomization of the label in the glucosyl moieties of glycogen when the substrate provided is galactose than when it is glucose

It has long been known that liver can produce glycogen twice as rapidly from fructose as from glucose In traversing the liver little fructose escapes into the general circulation and there is little hyperglucemia [53] This curious circumstance is probably attributable to the advantages possessed by the specific fructokinase over hepatic glucokinase

Hers [54] has shown that fructose incorporation into glycogen involves splitting of hexose into trioses and recondensation of the fragments In vivo hepatic glucokinase activity appears to be relatively weak and this important and critical phase of hepatic glycogen formation and its regulation remain relatively mysterious If glucose 6 phosphate is the initial product of hepatic kinase activity and if fructo e and hexose derived from gluconeogenesis must pass through glucose 6 phosphate on their way to glycogen it is not clear how this glucose 6 phosphate escapes the action of glucose 6-phosphatase Partition either physical or functional of glycogen formation and degradation becomes an attractive hypothesis

*Phosphoglucomutase*

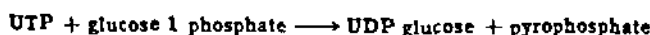
Presumably during conditions of glycogen synthesis sufficient glucose 6-phosphate accumulates within the cell to force formation of glucose 1 phosphate against the unfavorable 19 1 glucose 6-phosphate glucose 1 phosphate equilibrium of this enzyme

*Phosphorylase*

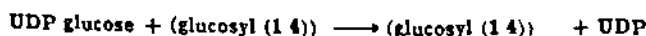
In vitro in the presence of a primer polysaccharide and at pH 7 0 this enzyme will add glucosyl units in $\alpha$ 1 4 linkage to the outer chains of the primer provided there is more than 1 mole of glucose 1 phosphate per 3 moles of inorganic phosphate in contact with the enzyme Recent work by Larner et al [55] demonstrated formation of glycogen by insulin timulated rat diaphragms in short time experiments despite a highly unfavorable ratio of inorganic phosphate to glucose 1 phosphate of 30

*UDPG-glycogen transglucosylase*

This enzyme discovered in liver by Leloir [9] and detected in muscle by Villar Pala i [56] and others [10 57 58] provides a pathway to glycogen independent of pho phorylase The steps involved may be summarized


UTP + glucose 1 phosphate ⟶ UDP glucose + pyrophosphate

The enzyme UDP gluco e pyrophosphorylase catalyzing this reaction is abundant in liver red blood cells mammary gland and mu cle


UDP glucose + (glucosyl (1 4)) ⟶ (glucosyl (1 4)) + UDP

This enzyme [11] variou ly termed UDPG glycogen tran glucosylase and UDPG glycogen synthetase requires a poly accharide primer The glucose re idue becomes linked α-1 4 to the polysaccharide The reaction is strongly activated by hexose 6-pho phate and no intermediary formation of a disaccharide occurs The liver enzyme can catalyze the tran fer of 100 µmoles of glucose from UDPG per hr per gm ti ue and the mu cle

enzyme 220 μmoles These values comfortably exceed the observed rates of 30 μmoles and 5 to 17 μmoles for glycogen formation in vivo in the two tissues The thermodynamics of the reaction are highly favorable for conversion to glycogen and transfers of the order of magnitude of 99 per cent have been observed [10] Mommaerts et al [59] envision that normally in muscle glycogen is synthesized at a certain rate by this enzyme but is kept at a moderate level by periodic decreases whenever glycogenolysis increases in association with exercise When muscle work is performed phosphorylase *b* is activated to phosphorylase *a* They further postulate that other factors besides the presence of this enzyme are in operation to limit the amount of glycogen which can be deposited in muscle

The discovery of this enzyme has made possible an understanding of the phosphorylase-deficient type of glycogen deposition disease but it has not entirely clarified the dilemma concerning the incongruities of various circumstances of glycogen breakdown and synthesis alluded to in the discussions of phosphoglucomutase and hexokinases In effect it has pushed back consideration of these problems at least one enzymatic step in that it makes glucose 1 phosphate the key compound in the ebb and flow of glycogen regulation The further partitioning of the processes of formation and breakdown in vivo is still an attractive hypothesis The activating effect of glucose 6-phosphate on this enzyme is intriguing in that it provides another possible mechanism for regulation of the flow of biochemical events

*Amylo (1 4 → 1 6) transglucosidase*

When a peripheral chain of a growing glycogen molecule reaches between 7 and 21 glycosyl units by means of successive transfers from UDPG (or phosphorylase action) the transglucosidase transfers a string of as yet undetermined number of 1 4 linked glucosyl units from their proximal 1,4 attachment on the main chain to a 1 6 attachment to the same or another unit of the main chain allowing renewed 1 4 additions to the bared end [60] An arborized structure results and the new 1 6 link constitutes a branch point The enzyme has been referred to as brancher'

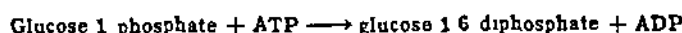
The thermodynamic properties of this enzyme have not been described but it is generally accepted as being irreversible and the specific debrancher amylo-1 6-glucosidase previously discussed is necessary to undo the 1,6 linkage formed (Fig 6-4)

*Accessory Enzymes*

Two enzymes have been found in animal tissues which conceivably could influence the accumulation or dissipation of glycogen by regulating the availability of glucose 1 6-diphosphate for the phosphoglucomutase reaction These enzymes have not been extensively studied and little

information concerning their physiologic roles if any is available These are

*Glucose 1 phosphate kinase* [61] which catalyzes the reaction



Glucose 1 phosphate + ATP ⟶ glucose 1 6 diphosphate + ADP

*Hexose 1 6-diphosphatase* [62] which catalyzes the reaction



Hexose 1 6 diphosphate ⟶ hexose 6 phosphate + phosphate

## FACTORS REGULATING THE CONCENTRATION OF GLYCOGEN IN THE TISSUES

New details concerning the regulatory factors for tissue glycogen have been added since the comprehensive review by Recant [63] Since diagnostic procedures and therapeutic attempts have been based on such factors they will be described briefly

### *Nutrition*

Liver glycogen is a principal reserve source of blood glucose Rapid depletion occurs in fasted animals Diets high in carbohydrate especially those high in fructose or fructose sources [64] rapidly replete the liver glycogen Excessive deposition of liver glycogen due to hyperalimentation in the normal subject has not been described There appears to be a finite limit in the normal animal to the amount of glycogen which can be stored and when this is reached carbohydrate is diverted to fat synthesis and stored as such in the adipose depots Approximately 45 gm glucose is available to a fasting man from liver glycogen Under basal conditions of peripheral glucose utilization this would not suffice to maintain normoglycemia for more than a few hours unless supplemented by glucose from gluconeogenesis

In the muscles and other peripheral tissue any substance which increases glucose uptake from the extracellular fluid and circulating plasma increases tissue glycogen levels and materials which inhibit glucose uptake have the opposite effect During sustained vigorous muscle contractions glycogen is rapidly consumed This occurs despite enhanced entry of glucose from the blood [65] which also attends muscle work and persists for a considerable time after resting conditions have been reestablished thereby facilitating replenishment of glycogen stores

### *Insulin*

Experiments employing incubation of the isolated rat diaphragm clearly prove that glucose uptake and glycogen synthesis are strikingly enhanced by insulin [66] Stadie and coworkers [67] have presented evidence that enhancement of muscle glucose utilization by insulin is primarily achieved by increased glycogen formation

The problem of a direct action of insulin on hepatic glycogen formation is confusing and in some doubt Positive effects on net glycogen synthesis in vitro have been denied consistently but enhanced incorporation of radiolabel into glycogen from glucose $C^{14}$ supplied in the medium has been observed [68] Renold et al [69] have found positive insulin effects in vivo It is well established that insulin therapy in uncontrolled diabetes and in animals experimentally deprived of insulin causes repletion of low liver glycogen stores The effect is not immediate and takes a matter of hours This is reminiscent of the time requirement for the adaptive changes in glucose 6-phosphatase activity levels It may be that insulin action on the liver involves adaptive changes in one or more enzymes of glycogen formation and that the effect is not a direct immediate one as it is on the penetration of glucose into muscle cells

The usual sequence of events following exogenous insulin administration to an intact normal animal is an immediate increase of glucose uptake from the blood by the muscle and increased glycogen in these tissues, the blood sugar falls and liver glycogen is mobilized in defense Later if an adequate glucose supply is available liver glycogen also rises

*Adrenal Steroids*

The 11 and 11 17-oxysteroid hormones of the adrenal cortex have a profound influence on liver glycogen Before specific chemical methods were perfected bioassay of these hormones depended on the ability of the test substance to promote storage of liver glycogen in adrenalectomized animals [70] Since no immediate direct enzymatic effect on hepatic glycogen economy has been described the enhancement of glycogen deposition seems best related to ability of these steroids to increase gluconeogenesis from protein Recent evidence suggests that this action is probably related to stimulation of hepatic transaminase activity [71] Other evidence has been presented that these hormones decrease the glucose uptake of muscle and secondarily decrease muscle glycogen synthesis [72]

The combined effects of increasing liver glucose output through stimulation of gluconeogenesis and decreasing peripheral uptake of glucose are thought to account for the ability of these steroids to defend the adrenalectomized animal from hypoglycemia and to produce diabetes mellitus when given or elaborated in excessive amounts The relatively long lapse of time required for effects to become apparent in vivo and the inability to obtain distinct carbohydrate changes in vitro suggest that adaptive rather than direct mechanisms are involved

*Somatotropin and Other Pituitary Factors*

In the presence of adequate insulin somatotropin promotes accumulation of liver glycogen spares carbohydrate from combustion and favors

protein synthesis and accretion by shifting metabolism toward the mobilization and utilization of lipid As with glucogenic adrenal steroid liver glycogen storage is permitted and enhanced and liver glucose output increased but the critical difference in the two influences is that the pituitary factor accomplishes these effects at the expense of fat while the adrenal hormones do so at the expense of protein An inhibitory effect of pituitary factors on extracted muscle hexokinase has been reported The prevention of this inhibition by added insulin constitutes the evidence for a useful hypothesis concerning a mechanism of insulin action [73 74]

Glycogenolysis in the beef adrenal cortex is enhanced in vitro by the presence of ACTH in the medium [75] Haynes has demonstrated that the effect is mediated through an accumulation of adenosine-3, 5 monophosphate within the tissue and an increase in active phosphorylase The situation appears to be analogous to the activation of liver phosphorylase by glucagon and epinephrine Haynes suggested that the activation of adrenal cortex phosphorylase by ACTH could be the mechanism by which the pituitary hormone stimulates steroid production for he points out that glycogenolysis would stimulate the glucose 6-phosphate dehydrogenase system and provide an abundance of reduced TPN required for corticosteroid biosynthesis

*Glucagon and Epinephrine*

Both glucagon and epinephrine are powerful activators of hepatic glycogenolysis and produce significant hyperglycemia when injected into animals Their glycogenolytic action in the liver is the basis of an important diagnostic test in glycogen deposition diseases It is a curious paradox that the exact enzymatic mechanisms of action in glycogen metabolism have been better worked out for glucagon than for any other known humoral agent and yet there is no firm hypothesis concerning its physiologic role or importance The enzymatic changes due to epinephrine as regards glycogen breakdown are identical with those of glucagon with the exception that epinephrine works in muscle enzyme systems as well as in the liver Glucagon stimulation of glycogenolysis is confined solely to the liver [28] and glucagon possesses none of the vasomotor regulating properties of the catecholamines of the adrenal medulla and sympathetic ganglia Despite the similarity of the enzymatic mechanisms of action of the two Cornblath [76] could not obtain additive results when liver slices were incubated in concentrations of the agents which independently gave responses of 50 per cent or less of maximum

Circumstantial but almost conclusive evidence has pinpointed the alpha-cells of the islets of Langerhans as the source of glucagon [77] If more than 75 per cent of these cells are destroyed by the administration of cobalt salts before a pancreas is extracted the hyperglycemia

producing properties of the extract are lost [78] Attempts at crystalliza tion of this hyperglycemic factor have been successful [79] It is a zinc free polypeptide with 29 amino acid components [79a] differing from insulin by containing methionine and tryptophan and by lacking proline iso-leucine and cystine found in the latter The problem of glucagon inacti vation was attacked by Goldner et al [80] by perfusing the material through isolated liver They reported that passage through the liver is attended by a loss of hyperglycemia provoking activity even though adsorption during passage does not take place Williams and coworkers [81] have evidence that glucagon insulin and corticotropin share the same deactivating enzyme system in liver Hill and Smith [82] have sug gested that leucine aminopeptidase detected in the submicroscopic par ticles of rat liver homogenates is an important enzyme in the degradation of the polypeptide since they were able to demonstrate complete rapid and facile hydrolysis of crystalline glucagon by this enzyme in vitro

The potency of the crystalline material is impressive 0 7 μg per kg is sufficient to produce a 50 per cent increase in blood sugar Administration of insulin sufficient to give a fall in blood sugar of the same magnitude requires a twenty five-fold larger dose on a molar basis Hyperglucemia seldom persists for as long as 60 min following a dose of glucagon Ad ministration is not attended by tachycardia blood pressure rise or in creased release of lactic acid from the muscles All these phenomena occur regularly when doses of epinephrine sufficient to provoke equivalent hyperglycemia are used Further purified glucagon can be given sub-cutaneously intramuscularly or intravenously with equal safety and approximately equal effectiveness in contrast to epinephrine nor epinephrine or other sympathomimetic amines An attendant phenome non which has been observed is that simultaneously administered sym patholytic agents counteract the hyperglycemogenic actions of epinephrine but not of glucagon [83] Obviously a positive hyperglycemic response to glucagon or epinephrine presupposes an adequate supply of mobilizable liver glycogen

The interchange between active phosphorylated liver phosphorylase and inactive dephosphophosphorylase and the depolymerization of stored glycogen has already been described It is via changes in this interplay that glucagon and epinephrine effect their glycogenolytic action As a result of the series of brilliant contributions by Sutherland and coworkers [12–14 23 24] the mechanisms of glycogenolytic action of these two humoral agents can be described in detail

1 Incubation of liver with glucagon or epinephrine results in the appearance of a heat stable dialyzable nucleotide containing adenine ribose and phosphate in molar ratios of 1 1 1 This has been tentatively identified as adenine ribose 3 5 phosphate

2. In the presence of this cofactor and ATP the proportion of phosphorylase in the active phosphorylated form rises sharply. It is not clear *whether this increase in the active form is the result of stimulation of the* dephosphophosphorylase kinase activity or of inhibition of the deactivating liver phosphorylase phosphatase.

3. The net increase of active phosphorylase results in an increased rate of glycogenolysis and subsequent increased release of free glucose. Increase in glycogen synthesis as a result of glucagon or epinephrine activation of phosphorylase has not been observed.

4. In the intact animal with normal islet cell function hyperglycemia induces insulin release. The ability of epinephrine to promote muscle glycogenolysis, a phenomenon not shared with glucagon, modifies the insulin release response in the periphery and explains the rise in serum pyruvate levels after epinephrine hyperglycemia but not after glucagon hyperglycemia [84]. This difference in action in muscle also serves as an adequate explanation for the failure to observe a fall in serum inorganic phosphate concentration after epinephrine hyperglycemia. A fall is seen routinely after glucagon hyperglycemia [79].

It is of interest that sympathomimetic amines other than L-epinephrine can reactivate inactive liver phosphorylase and thereby enhance glycogenolysis. Presumably the reactivation with these substances proceeds through the intervention of the same cofactor nucleotide. Table 6-1, a

TABLE 6-1 RELATIVE LIVER PHOSPHORYLASE-REACTIVATING ACTIVITIES OF SYMPATHOMIMETIC AMINES IN VITRO AND IN VIVO

| *Sympathomimetic amine* | *Relative liver phosphorylase assay* | *Activity* intact animal assay* |
|---|---|---|
| L-Epinephrine | 100 | 100 |
| L-Norepinephrine | 10 | 1[illegible] |
| D-Epinephrine | 1 | |
| D-Norepinephrine | 0.4 | 0.6 |
| DL-Amphetamine | 0.000[illegible] | 0.0 |

* Potency of compound is relative to that of L-epinephrine calculated on a molar basis.

SOURCE: Modified from T. W. Rall et al. [15].

modification from Sutherland's report [15], indicates the relative activities of these amines and their isomers and shows the close correlation of homogenate assays with intact animal assays. This is good evidence that the enzymatic processes studied were those responsible for the actions of these compounds in normal physiology. The hyperglycemia seen in cases of pheochromocytoma is undoubtedly explained by the low but definite glycogenolytic potentialities of L-norepinephrine.

*Thyroid Hormone*

One of the consequences of excessive L-thyroxine administration is the gradual depletion of liver glycogen and increase in glucose lactate and pyruvate in the blood [85] Similar changes are seen in patients with hyperthyroidism The biochemical and enzymatic effects of this hormone are considered elsewhere in this volume (Chap 9) The most plausible explanation for the influence of L thyroxine on glycogen metabolism is that the depletion results from draining off of hexose and other carbon sources into the energy production systems of the Krebs cycle which have been rendered inefficient because of the uncoupling of oxidative phosphorylation The uncoupling of oxidative phosphorylation by L-thyroxine is discussed elsewhere [86] (Chap 9) The hormone has been tried therapeutically in glycogen deposition diseases apparently with partial success in some cases

*Ovarian Steroids*

Progesterone induces an increase in glycogen content of the stromal cells of estrogen provoked proliferative endometrium as they undergo transition into the secretory and decidual phases of their normal cycle No information is available as to how this stimulus to glycogen accumulation is biochemically mediated but the phenomenon is cited as an indication that glycogen metabolism in highly specialized tissues may be modified by and in intimate relationship with special regulating influences

*Diseases*

The low glycogen content of some cirrhotic livers may be related to declining enzyme concentrations which occur as the disease reaches its advanced states In diabetes in spite of low liver content when the disease is uncontrolled increased glycogen may be found in the kidney pancreatic islets and cardiac muscle [8, 87] Deposition of glycogen in the pigment epithelium of the iris a tissue which normally has little or no histochemically demonstrable glycogen has also been noted in diabetic patients [88]

Perhaps these anomalous depositions are the result of the hyperglycemia They serve as suggestions that the glycogen metabolism of specialized tissues must be characterized independently

## DISEASE STATES RESULTING FROM PRIMARY ABNORMALITIES IN GLYCOGEN METABOLISM

Gerty Cori deplored the practice of treating all glycogen deposition diseases as a single *uniform disease* she recommended a classification based upon specific causative enzyme defects [3] She also pointed out

that naming the entities by the predominant organ of excessive glycogen content leads to misconceptions and expressed a preference for a nosology consisting of serially numbered types While a numbering system allows no criticism on semantic grounds for present purposes it is convenient to modify and extend Cori s classification in order to indicate the specific enzymatic step which is at fault in each of the various glycogen storage diseases which has been elucidated (Table 6-2)

TABLE 6-2 TYPES OF GLYCOGEN DEPOSITION DISEASES (GLYCOGEN THESAUROSE )

| Cor typ | E ym d f t | O g | Gly g t t | Ep ym m | S g sted l l m |
|---|---|---|---|---|---|
| 1 | Gl cose 6-pl ph t se | Li k l | N l | G k d se | Cl os C 1 t os hat se d fi y h j to- r l gly se |
| 2 | ? | G d | N m l | P mp d | ... glyg |
| 3 | Amylo-1 6- l os l se (t l ) | Li h t m l | Ab m l ss ry l t | F b d se | D b l d f y l m t d xtri |
| 4 | Amylo-(1 4 → 1 6) t gl l se (b l ) | L h bly tl g | Ab m l l g d te b h | A l se d se | B h d fi y myl pe t os |
| 5 | M l ph l l se | M l | N l | M A dle- S h l P d sease | My phosph ryl d fi y gly o- s |
| 6 | I yl | Li | N l | H rs d s se | H t t sph ryl d fi y gly g o- |

It should be emphasized that this system of classification is not permanent or necessarily the most advantageous clinically The classifications of Chaptal et al [89] of Zellweger [90] and of Andersen [91] take more cognizance of variations in clinical manifestations in case where enzyme defects have not been apparent Citzelmann [92] in his outstanding and exhaustive review of the glycogen deposition disease problem and its relationship to glucagon prefers a functional classification into two groups the first with a good response and the second without a normal response to glucagon At the time his proposal was made all forms of the disease with generalized glycogen storage normal enzyme and normal glycogen fell into the glucagon responsive category and all forms with enzyme deficiency fell into the abnormally responsive category More recently the description of myophosphorylase deficiency glycogenosis in which the glucagon response is normal and the enzyme defect and excessive glycogen deposition are present in the skeletal muscles only tends to make the classification by glucagon response confusing and restrictive Therefore it seems best to follow Cori's system extending it as specific etiologic mechanisms are delineated and

emphasizing differences in specific etiology rather than functional or clinical variations

GLUCOSE 6 PHOSPHATASE DEFICIENCY HEPATORENAL GLYCOGENOSIS

Synonyms von Gierke's disease type I of Cori hepatorenal form, hepatonephromegalia glycogenica van Creveld von Gierke's disease

In retrospect it is probable that the examples of children with stunted growth and hepatomegaly described by several authors [93-96] were

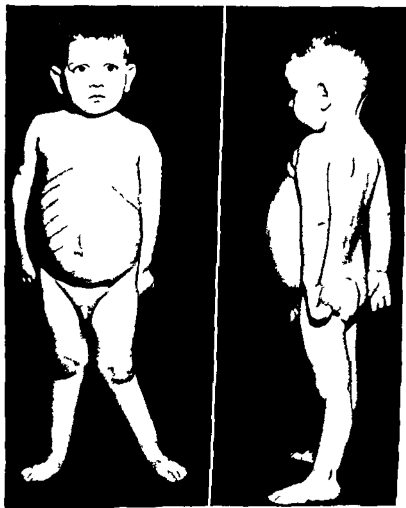


Fig 6-5 G I a 6-year-old patient with biochemically proved absolute deficiency of hepatic glucose 6-phosphatase The liver contour has been outlined with a marking pencil Xanthomas present over the elbows knees and buttocks cannot be discerned in the photograph Note the characteristic lumbar lordosis abdominal contour and sufficiency of depot fat accumulation

instances of the disease first described pathologically by von Gierke in 1929 [97] Since von Gierke's description numerous articles and reviews have appeared which have given this disease a full and extensive characterization The interested reader is referred to Gitzelmann's paper [92] for a complete listing of these authors and the contributions of Bischoff [98] Schonheimer [99] Kimmelstein [100] Wagner [101] and Van Creveld [102 103] especially are recommended

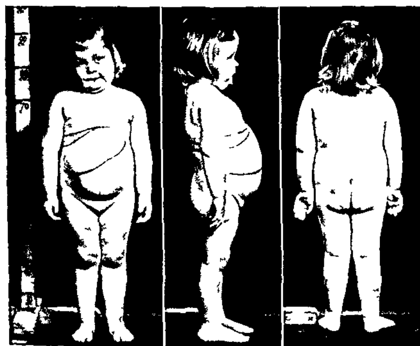


Fig 6-6 P D a 4 year-old patient with biochemically proved absolute deficiency of hepatic glucose 6-phosphatase The accumulation of depot fat appears more than adequate

Symptoms and recognizable clinical signs usually appear in the first year of life and hepatomegaly may be present at birth Convulsions due to hypoglycemia failure to thrive episodes of precipitous severe acidosis and eruptive xanthomas are the most frequent manifestations History of an older afflicted sibling should arouse suspicion and the disease should be searched for at birth in all subsequent offspring of the same mating

Physical findings in a typical case usually include (Figs 6-5 6-6)

1 Retarded growth without disproportion of the head limbs or trunk length

2 Tendency to adiposity with notable accumulations in the depots of the cheeks, breasts buttocks, and the backs of the arms and thighs

3 Immense abdominal rotundity especially noticeable in nurslings and small children due to a massively enlarged liver The blunt hard edge may extend as low as the level of the iliac crest The spleen is not palpable and the liver not tender in typical cases

4 The skin usually has a yellowish and pallid hue and pinkish yellow xanthomas on the elbows knees shins or buttocks may be present [10i]

5 The gait is typically swinging and on a wide base with at times accentuation of lumbar lordosis in order to accommodate the massive liver and abdomen

6 If abdominal examination can be adequately performed enlargement of the kidneys may be apparent

Laboratory examinations in cases of some duration reveal many or all of the following findings

1 Marked and at times profound fasting hypoglycemia (0 to 15 mg per 100 ml true blood glucose) In many cases there appears to be a clinical tolerance to hypoglucemia, perhaps because of some "adaptation" One is frequently amazed by the absence or paucity of clinical signs and symptoms of hypoglycemia in the presence of blood glucose concentration reduced to a point where convulsions and unconsciousness would be expected in a normal child Normal electroencephalograms were found in two cases studied by Schulman and Saturen [10o] in the presence of very low blood sugar levels Postcibal blood sugar levels tend to be high and values in the first hour after a carbohydrate feeding may be in the diabetic range

2 Marked hyperglyceridemia hypercholesterolemia and hyperfatty acidemia Frequently the content of fatty solids in the serum reaches 10 per cent or more by volume Indeed a proportional correction must be made in order to determine the true aqueous concentrations of various dissolved components of the serum For example in a case where the total plasma solids were approximately 15 per cent by volume application of a correction factor of 1 18 was necessary this factor when applied to the determined serum sodium concentration of 119 mEq per liter yielded a perfectly normal value of 140 mEq per liter If this volume correction is not taken into account when hyperlipemia is massive false impressions of depressed electrolytes NPN $CO_2$ etc will be obtained from direct serum determinations (Fig 6 7)

3 Acetonuria and acetonemia These are inconsistently present and appear to be related to episodes of acidosis and fasting It should be mentioned that several cases have been seen in which severe life threatening acidosis supervened without the appearance of significant acetonemia or acetonuria the absence of ketone bodies therefore cannot be used as reliable assurance that acidosis is not present or impending

4 Mild anemia in the range of 8 0 gm per 100 ml has been seen in several cases Extensive hematologic investigation by McGovern [106] in one ca e failed to define a causative mechanism

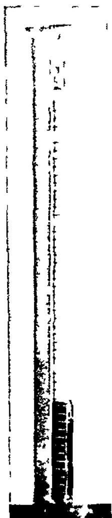


Fig 6– H matocrit d t mn ti n on pa t nt ( l l wing inten e l p l ma of erum n l lw l m atocrit The total pla ma s l l were approximat ly l per cent by v l ume at the t n of th l t rmi nation

5 Elevations of serum lactate and pyruvate levels have been *frequent in some cases* even when acidosis was not present and although they rose significantly in one case when acidosis occurred the additional amount was much too small to account for the bi carbonate which had been displaced

6 Absolute depression of the serum inorganic phosphate and normal serum alkaline phosphatase activity are frequently observed and may correlate with the generalized decreased bone density en countered on roentgenographic examination of the skeleton The effect of chronic metabolic acidosis may also play a role in this finding

Mushrooming of the metaphyses and small epi phy eal growth centers also has been described A feature of one patient at age 6 was the repeated occurrence of leg fractures following minim al trau ma and markedly delayed healing

7 Glucosuria and a nonspecific aminoaciduria *may occur and at times may be massive [107]* It has been suggested that the severity of this finding correlates with the everity of glycogen infiltration of the renal tubules Aside from this abnormality it is surpri ing that even with much enlargement of the kidneys little imp urment of renal function can be detected

8 Liver function tests other than tho e of carbo hydrate metaboli m al o fail to show much deviation from the norm il The albumin globulin ratio is de pres ed in only about half the cases Electrophoretic tudies *[89] in three ca es showed increases in* $\alpha_2$ and $\beta$ globulins along with incre e es in $\alpha_2$ and $\beta$ lipoprotein and $\alpha_2$-mucopoly accharide

9 The electrocardiogram is u ually normal but may show changes during epi ode of profound hypoglycemia [108]

10 Increases in the fraction of nonfermentable reducing ubstances in the whole blood have been reported [109] and were hown to be due to accumu lation of phosphorylated glycolytic intermediate chiefly glucose 6-pho phate fructose 6-pho ph ite

and hexose diphosphate Elevations of total glycogen content of whole blood were also noted Apparently these substances are in the circulating blood cells since it has not been possible to detect their presence by specific enzymatic assay in the serum [47]

*Functional Tests*

Further useful information in a suspected case of this disease can be obtained by performing functional tests of carbohydrate metabolism The glucose tolerance test may show an abnormally high rise and in some cases a delayed fall Caution should be used in performing this test in that an overnight fast may provoke hypoglycemic convulsions or unconsciousness Fructose and galactose tolerance tests are said to give flat or normal curves In a relatively compensated child with this disorder nitrogen balance is usually normal Insulin tolerance tests are to be avoided in view of inability of the patient to defend himself against hypoglycemia

Most important are the responses to the glycogenolytic properties of glucagon and epinephrine By the mechanisms previously described in the section on liver phosphorylase activation administration of these agents in a well nourished normal child with a well glycogenated liver results in 40 to 60 per cent rise in blood glucose within 10 to 20 min by virtue of the depolymerization of stored glycogen to glucose 1 phosphate subsequent conversion to glucose 6-phosphate and ultimate release into the hepatic vein blood after dephosphorylation of free glucose and inorganic phosphate In biochemically verified examples of this disease the hyperglycemogenic response to these agents is abolished or decidedly diminished The severe hypofunction of glucose 6-phosphatase found in this disease limits the amount of glucose 6-phosphate which can be deesterified even in the face of a brisk rise in intracellular glucose 6-phosphate concentration

It will be recalled that as much as 8 per cent of the glucose of a glycogen molecule can be released as free glucose by debrancher action (amylo-1 6 glucosidase) without need of the specific phosphatase Accordingly some increase in peripheral blood glucose is to be expected even in the total absence of glucose 6 phosphatase This is a reasonable explanation for the clinical observations that in a few cases of what appear to be classical glucose 6-phosphatase deficiency disease only slightly diminished or approximately normal responses have been found [110] Partial responses might also be expected in those cases in which glucose 6-phosphatase activity is less severely diminished

It is conceivable that near normal responses to the two hyperglycemic factors which have been observed in cases thought to have been glucose 6-phosphatase deficiency disease on clinical grounds resulted from con

fusion with debrancher deficiency limit dextrinosis (see Amylo-1 6 glucosidase above) In the latter condition after a recent feeding there may be considerable transient elongation of the terminal branches of the glycogen molecule which makes possible a substantial rise in blood sugar when depolymerization is promoted by increasing active phosphorylase In some cases direct biochemical investigation of liver tissue in regard to glycogen structure and the activity levels of glucose 6 phosphatase and amylo-1 6 glucosidase is necessary in order to differentiate the two conditions with absolute certainty

Suffice it to say that marked resistance to the hyperglycemic actions of glucagon and epinephrine is a finding lending strong support to the diagnosis of hepatic glycogenosis but a normal response does not absolutely rule it out Of the two the glucagon test is the more useful because of avoidance of the vasomotor effects of epinephrine and the occasional confusing interference with muscle carbohydrate metabolism Since nonantigenic and nonpyrogenic preparations of glucagon are now available in a lyophilized form for long storage and easy solubilization it is customary to give 1 0 mg crystalline glucagon intravenously to a subject after an 8-hr fast Determinations of fasting 10- 20- 30- 40- and 60-min true blood glucose are made When it seems advisable the duration of the preliminary fast period is reduced

Another functional test useful in diagnosis was suggested by Schwartz et al [111] One gram of galactose per kg body weight is infused intravenously over a 3 min period and preinjection 1 10- 20- 30- 45- and 60-min blood samples are obtained and analyzed for glucose and reducing sugars

Hers and Malbrain [112] have shown that fructose 0 5 gm per kg body weight can be used instead of galactose In a normal child an elevation of blood glucose occurs after such infusions but in glucose 6-phosphatase deficiency disease the inability of the liver to deesterify glucose 6-phosphate formed by intrahepatic conversion of the administered sugar results in a failure of blood glucose to rise The occurrence of hyperglycemia after such a test implies quantities of glucose 6-phosphatase in the liver sufficient for the needs of the organism Normal hyperglycemic responses with this test have been obtained in debrancher deficiency limit dextrinosis [11?] which is the expected response in that no defect in the critical phosphatase is present The two conditions can be distinguished on this basis when other tests and manifestations are equivocal The assimilation of galactose and fructose and their interconversion to glucose 6-phosphate by the liver is normal in the uncomplicated case of glycogenosis However the utilization of galactose is more easily disturbed than is the utilization of fructose when other forms of liver disease are present Caution with the infusion of fructose is also necessary

and hexose diphosphate. Elevations of total glycogen content of whole blood were also noted. Apparently these substances are in the circulating blood cells, since it has not been possible to detect their presence by specific enzymatic assay in the serum [47].

*Functional Tests*

Further useful information in a suspected case of this disease can be obtained by performing functional tests of carbohydrate metabolism. The glucose tolerance test may show an abnormally high rise and in some cases a delayed fall. Caution should be used in performing this test in that an overnight fast may provoke hypoglycemic convulsions or unconsciousness. Fructose and galactose tolerance tests are said to give flat or normal curves. In a relatively compensated child with this disorder, nitrogen balance is usually normal. Insulin tolerance tests are to be avoided in view of inability of the patient to defend himself against hypoglycemia.

Most important are the responses to the glycogenolytic properties of glucagon and epinephrine. By the mechanisms previously described in the section on liver phosphorylase activation, administration of these agents in a well nourished normal child with a well glycogenated liver results in 40 to 60 per cent rise in blood glucose within 10 to 20 min by virtue of the depolymerization of stored glycogen to glucose 1-phosphate, subsequent conversion to glucose 6-phosphate, and ultimate release into the hepatic vein blood after dephosphorylation, of free glucose and inorganic phosphate. In biochemically verified examples of this disease the hyperglycemogenic response to these agents is abolished or decidedly diminished. The severe hypofunction of glucose 6-phosphatase found in this disease limits the amount of glucose 6-phosphate which can be deesterified even in the face of a brisk rise in intracellular glucose 6-phosphate concentration.

It will be recalled that as much as 8 per cent of the glucose of a glycogen molecule can be released as free glucose by debrancher action (amylo-1,6-glucosidase) without need of the specific phosphatase. Accordingly some increase in peripheral blood glucose is to be expected even in the total absence of glucose 6-phosphatase. This is a reasonable explanation for the clinical observations that in a few cases of what appear to be classical glucose 6-phosphatase deficiency disease, only slightly diminished or approximately normal responses have been found [110]. Partial responses might also be expected in those cases in which glucose 6-phosphatase activity is less severely diminished.

It is conceivable that near normal responses to the two hyperglycemic factors which have been observed in cases thought to have been glucose 6-phosphatase deficiency disease on clinical grounds resulted from con

fusion with debrancher deficiency limit dextrinosis (see Amylo-1 6-glucosidase above) In the latter condition after a recent feeding there may be considerable transient elongation of the terminal branches of the glycogen molecule which makes possible a substantial rise in blood sugar when depolymerization is promoted by increasing active phosphorylase In some cases direct biochemical investigation of liver tissue in regard to glycogen structure and the activity levels of glucose 6-phosphatase and amylo-1 6 glucosidase is necessary in order to differentiate the two conditions with absolute certainty

Suffice it to say that marked resistance to the hyperglycemic actions of glucagon and epinephrine is a finding lending strong support to the diagnosis of hepatic glycogenosis but a normal response does not absolutely rule it out Of the two the glucagon test is the more useful because of avoidance of the vasomotor effects of epinephrine and the occasional confusing interference with muscle carbohydrate metabolism Since nonantigenic and nonpyrogenic preparations of glucagon are now available in a lyophilized form for long storage and easy solubilization it is customary to give 1 0 mg crystalline glucagon intravenously to a subject after an 8 hr fast Determinations of fasting 10- 20- 30- 40- and 60-min true blood glucose are made When it seems advisable the duration of the preliminary fast period is reduced

Another functional test useful in diagnosis was suggested by Schwartz et al [111] One gram of galactose per kg body weight is infused intravenously over a 3 min period and preinjection 1 10- 20- 30- 45- and 60-min blood samples are obtained and analyzed for glucose and reducing sugars

Hers and Malbrain [112] have shown that fructose 0 5 gm per kg body weight can be used instead of galactose In a normal child an elevation of blood glucose occurs after such infusion but in glucose 6-phosphatase deficiency disease the inability of the liver to dephosphorylate glucose 6-phosphate formed by intrahepatic conversion of the administered sugar results in a failure of blood glucose to rise The occurrence of hyperglucemia after such a test implies quantities of glucose 6-phosphatase in the liver sufficient for the needs of the organism Normal hyperglucemic responses with this test have been obtained in debrancher deficiency limit dextrinosis [112] which is the expected response in that no defect in the critical phosphatase is present The two conditions can be distinguished on this basis when other tests and manifestations are equivocal The assimilation of galactose and fructose and their interconversion to glucose 6-phosphate by the liver is normal in the uncomplicated case of glycogenosis However the utilization of galactose is more easily disturbed than is the utilization of fructose when other forms of liver disease are present Caution with the infusion of fructose is also necessary

because precipitous production of severe acidosis has occurred with this procedure in an enzymatically verified case of absolute glucose 6-phosphatase deficiency disease [47]

*Biochemical Studies*

The ultimate and only conclusive diagnostic procedure in glycogen deposition diseases is liver biopsy and direct biochemical study of the amount and structure of the glycogen along with assay of the levels of activity of the specific enzymes Sufficient tissue for such studies may be obtained by needle aspiration of the liver but since the procedure is a blind one most authorities prefer general anesthesia and surgical biopsy under direct vision The surgical approach obtains sufficient tissue for pathologic examination and diagnostic information is provided even if glycogen deposition disease is not present The principal hazard in surgical biopsy is the possibility of fatal outcome due to effects of general anesthesia on a glycogen infiltrated myocardium Occasionally such infiltration gives no clinical evidence of its existence as part of a generalized glycogenosis or limit dextrinosis The interested reader is referred to the paper of Hers [8] for a detailed description of the elegant methodology which he has worked out for the required enzymatic studies

The biochemical findings from enzymatic studies on liver and kidney tissue from examples of glucose 6-phosphatase deficiency glycogenosis were first reported by Cori and Cori [1 113] and have been amply confirmed by others [8 114] The following findings can be considered diagnostic

1 Glycogen content above 4 per cent by wet weight

2 Normal structured glycogen as determined by alternate phosphorylase and amylo-1 6 glucosidase stepwise degradation viz normal chain length and normal frequency of branch points

3 Normal or only slightly reduced levels of activity of phosphorylase amylo 1 6 glucosidase phosphoglucomutase UDPG glycogen transglucosylase and amylo-(1 4 ⟶ 1 6) transglucosidase The high content of glycogen makes it desirable to compare enzyme activities not only per unit weight but also per unit of deoxyribonucleic acid or per cell

4 Absent or very low glucose 6-phosphatase activity Hers expresses the opinion [8] that only total absence of the enzyme can be held responsible for the disease In other forms of hepatic disease moderately reduced glucose 6-phosphatase activity may be due to absolutely reduced content of the enzyme or dilution of protein content by fat or other infiltrations Apparently total or almost total absence of this enzyme is necessary for glycogen accumulation when other glycogenolytic enzymes are present at adequate activity levels In the experience of Hers [8] accumulation of glycogen can be attributed to other enzymatic deficiencies when glucose 6-phosphatase assays show only moderate scarcity of the enzyme

Hers further pointed out that total or severe reduction of glucose 6-phos phata e is required to impair the hyperglucemia following galacto e or fructo e infu ion

5 The total fat content of the liver is frequently much increased but this finding is not invariable and is not a *sine qua non* for the biochemical diagno is Increased renal fat content does not seem to occur but glycogen depo ition with increase in organ size may be extensive and total absence of glucose 6-phosphatase can be demonstrated

Microscopic findings are not diagnostic and consist of an abundance of glycogen granules in hepatic cells and in the cells of the convoluted tubules Fatty vacuolization of many liver cells and the variability of glycogen content from cell to cell combined with central placement of the nuclei give these cells a plant like swollen appearance when viewed in hematoxylin and eosin preparations In the typical case fibrosis ductile proliferation and destruction of lobular architecture characteristic of a cirrhotic process are not found

The epithelial cells of the convoluted tubules often protrude into the lumen to produce a scalloped effect The cells of the loops of Henle show imilar but less pronounced changes while the collecting tubules are usually normal Cellular debris found in the lumens contains some glycogen and may be in the form of casts In some cases increase in inter stitial fibrous tissue has been described

The histochemical technique of Chiquoine [115] beautifully demon strates the distribution of glucose 6-pho phatase within the cytopla m of normal hepatic cells and documents the absence of this enzyme in normal nuclei and stromal elements It also shows that there is a decreasing *gradient of enzymatic activity extending from the periphery of the normal* liver lobule toward the central vein In cases of glucose 6 phosphatase deficiency glycogenosis this same technique can be employed to demon strate in a most dramatic and graphic manner the absence of the enzyme (Fig 6-8)

Best s stain can be employed to confirm large deposits of glycogen in the liver and kidneys and only small amounts in the heart tongue and skeletal mu cle [116]

The hyperlipidemia in which all fractions of the blood lipids participate is a characteristic finding in biochemically verified cases of glucose 6-pho phatase deficiency glycogenosis Because the high level of serum lipids and lipoproteins in this *condition grossly re emble those seen in* uncontrolled diabetes mellitus it has been suggested that the stimulus to hyperlipidemia may be the same in the two diseases By the same analogy massive stimulation of ketogenesis has been an attractive hypothesis in accounting for the proclivity to severe acidosis in gluco e 6-phosphatase glycogenosis However there are some impressive in congruities in the two conditions which indicate that much more study

because precipitous production of severe acidosis has occurred with this procedure in an enzymatically verified case of absolute glucose 6-phosphatase deficiency disease [47]

*Biochemical Studies*

The ultimate and only conclusive diagnostic procedure in glycogen deposition diseases is liver biopsy and direct biochemical study of the amount and structure of the glycogen along with assay of the levels of activity of the specific enzymes Sufficient tissue for such studies may be obtained by needle aspiration of the liver but since the procedure is a blind one most authorities prefer general anesthesia and surgical biopsy under direct vision The surgical approach obtains sufficient tissue for pathologic examination and diagnostic information is provided even if glycogen deposition disease is not present The principal hazard in surgical biopsy is the possibility of fatal outcome due to effects of general anesthesia on a glycogen infiltrated myocardium Occasionally such infiltration gives no clinical evidence of its existence as part of a generalized glycogenosis or limit dextrinosis The interested reader is referred to the paper of Hers [8] for a detailed description of the elegant methodology which he has worked out for the required enzymatic studies

The biochemical findings from enzymatic studies on liver and kidney tissue from examples of glucose 6-phosphatase deficiency glycogenosis were first reported by Cori and Cori [1 113] and have been amply confirmed by others [8, 114] The following findings can be considered diagnostic

1 Glycogen content above 4 per cent by wet weight

2 Normal structured glycogen as determined by alternate phosphorylase and amylo-1 6-glucosidase stepwise degradation viz normal chain length and normal frequency of branch points

3 Normal or only slightly reduced levels of activity of phosphorylase amylo-1 6-glucosidase phosphoglucomutase UDPG glycogen transglucosylase and amylo-(1 4 ⟶ 1 6) transglucosidase The high content of glycogen makes it desirable to compare enzyme activities not only per unit weight but also per unit of deoxyribonucleic acid or per cell

4 Absent or very low glucose 6 phosphatase activity Hers expresses the opinion [8] that only total absence of the enzyme can be held responsible for the disease In other forms of hepatic disease moderately reduced glucose 6 phosphatase activity may be due to absolutely reduced content of the enzyme or dilution of protein content by fat or other infiltrations Apparently total or almost total absence of this enzyme is necessary for glycogen accumulation when other glycogenolytic enzymes are present at adequate activity levels In the experience of Hers [8] accumulation of glycogen can be attributed to other enzymatic deficiencies when glucose 6-phosphatase assays show only moderate scarcity of the enzyme

glucose 6-phosphate which would normally have been released after deesterification as free blood glucose but instead is channeled through the Embden Meyerhof pathway When the capacity of the Krebs cycle to accept and further di ..imulate the three carbon fragments has been exceeded pyruvate and lactate are delivered into the hepatic outflow Theoretically, the hexose monophosphate oxidative pathway for disposition of gluco e 6-pho phate would also be operating at maximal activity under the stimulus of a much increased substrate load These circum tances if present would be highly favorable for increased hepatic synthe is of fatty acid Therefore it is conceivable that hyperlipidemia in this condition is the result in part of overproduction in the liver rather than overmobilization from the depots

The theoretical considerations of the hyperlipidemia and the genesis of acidosis as they occur in this disease make it clear that faulty biogenetics involving the lack of one critical enzyme has provided an individual in whom by means of suitable investigation elucidation of the in vivo interdependence and mechanisms of regulation of hepatic carbohydrate and lipid metabolism may be possible

*Genetics*

Exten ive information concerning the genetics of this form of glycogen depo ition disease is not available It has long been recognized that there is a marked frequency of occurrence in siblings and in offspring of con sanguineous matings and that the defect is detectable at or shortly after birth in an equal male-female distribution When the glucose 6 phosphatase deficiency was discovered there followed general acceptance that the mode of transmission is as a completely recessive trait involving a single autosomal gene [*117*] which endows the liver and kidney with the capacity to synthesize glucose 6 phosphatase As such this form of glyco genosis qualifies as an example of an inborn error of metabolism

Until recently detection of the carrier state was not possible since severe deficiencies of the enzyme are necessary before glycogen deposi tion hypoglycemia and secondary metabolic consequences occur Hsia and Kot [*118*] have studied the phosphorylated intermediates of the glycolytic cycle of the red cells of affected persons and their parents Their results show a statistically significant increa ed content of these compounds in the presumed heterozygotes when compared with normal controls Although the cause of increased phosphorylated glycolytic intermediate content in the affected homozygotes themselves is not understood the data indicate that the degree of increase is of approxi mately the same magnitude in the heterozygotes These findings tend to confirm that this di ease is a simple rece ive trait (Table 6 3)

Information concerning the appearance of glycogen and enzymes necessary for its metabolism during the development of the guinea pig

of the genesis of hyperlipidemia and acidosis is necessary before a hyper mobilization theory can be accepted For example one cannot help being impressed with the relative overabundance of stored adipose tissue in a child with this defect Since this occurs when hyperlipidemia is chronically massive were excessive mobilization the mechanism, would not eventual depletion of adipose depots rather than relative excess be

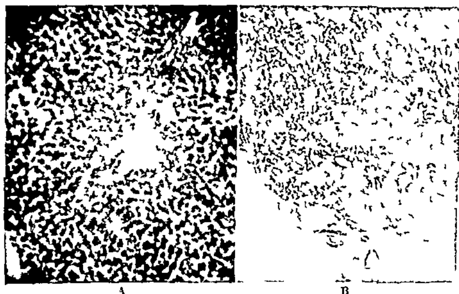



Fig 6-8 A Distribution of glucose 6-phosphatase in 10-μ frozen section of liver of a case of idiopathic generalized glycogenosis (type 2) The enzyme is present only within the cytoplasm of the hepatic cells and is absent from all nuclei and stromal elements This appearance corresponds with that seen in normal liver Note the gradient of enzymatic activity extending from the periphery of the lobule to the central vein (Light microscopy 85×) B Section of the liver from a case of glucose 6-phosphatase deficiency glycogenosis (type 1) No enzymatic activity can be demonstrated (phase contrast microscopy 100×)

more likely? The supply of glucose to the periphery is sharply curtailed by the hepatic enzyme deficiency What then is the primary substrate transported from the alimentary influx via the liver to the periphery for depot lipid synthesis?

Although excessive glycogen deposition in the liver can account for disposal of some alimentary carbohydrate through sequestration there appears to be a finite limit to how much the hepatic glycogen content can be increased Some other pathway of disposition of carbohydrate carbon in the liver seems probable Some of this carbon apparently is discharged from the glucose 6 phosphatase–deficient liver by increasing the production and release of pyruvate and lactate since elevated blood levels are routinely found Presumably this is the fate of some of the

Another puzzling question involved in fetal glycogen synthesis is that of the source of the initial primer molecules of the polysaccharide for both UDPG glycogen transglucosylase and phosphorylase as found in the animal after birth require the presence of such a primer for their synthetic action It is not known whether primer is transferred with the gametes or if there is a special system for its synthesis in fetal life It is also possible that these two enzymes can function without primer requirement during this period or that the initial primer is of maternal origin

*Therapy*

The question of therapy in glucose 6-phosphatase deficiency disease should not be regarded as hopeless With recognition of the metabolic mechanisms involved in the clinical manifestations it has been possible through relatively simple rational therapy to prevent death in early life It has been suspected that the disease becomes less severe in those who survive beyond the fourth year although hepatomegaly and severe reduction of enzyme activity remain unchanged [121]

During infancy severe hypoglycemia with its inherent threat of damage to the central nervous system is the central clinical problem A reasonable and effective approach to this problem is the institution of frequent feedings of carbohydrate throughout the day and night On theoretical grounds it seems advisable to avoid a great excess of carbohydrate The aim is to provide a relatively constant influx of blood glucose from alimentation without encouraging rapid increase in glycogen deposition The creation of a portocaval shunt might be very effective in this regard but no report of a trial of this procedure has been found

Since infection and acidosis are the usual proximate causes of death it behooves the clinician (1) to protect the child from them in so far as is possible and (2) to recognize their appearance quickly and institute corrective therapy Rehydration intravenous glucose vigorous antibiotic therapy if infection is found all employed with care and frequent laboratory determinations give results comparable to those in the treatment of diabetic ketoacidosis provided the situation has not deteriorated too far Administration of bicarbonate in suitable amount is effective in hastening the reversal of acidosis Alkali in the form of lactate seems inadvisable in view of the hyperlactic acidemia and the possibility of further exceeding the capacity for its utilization

The above methods of therapy although effective in preventing dangerous hypoglycemia and loss of life due to acute episodes of acidosis are strictly defensive and do not attempt to correct influence or by pass the underlying enzymatic defect Attempts to do so with pancreatic extracts hypophyseal extracts adrenocortical hormones epinephrine whole liver liver extracts choline cholic acid lecithin bile salts and

embryo indicate that the mechanisms of genic expression concerned in this area of metabolism are not simple and that secondary modifying influences may be at work According to Nemeth and coworkers [119]

TABLE 6-3 RED BLOOD CELL CONTENT OF PHOSPHORYLATED GLUCOSE AND FRUCTOSE IN NORMAL SUBJECTS, PATIENTS WITH GLUCOSE 6-PHOSPHATASE DEFICIENCY GLYCOGENOSIS AND THEIR PARENTS

| | *Normal subjects* | | *Affected patients* | | *Parents (heterozygotes)* | |
|---|---|---|---|---|---|---|
| | G 6-P | F 6-P | G 6-P | F 6-P | G 6-P | F 6-P |
| Number | 10 | 10 | 4 | 4 | 8 | 8 |
| Mean | 0 18 | 0 38 | 1 09 | 1 30 | 1 10 | 1 56 |
| Range | 0 05–0 3[illegible] | 0 12–0 [illegible] | 0 80–1 [illegible] | 1 15–1 [illegible] | 0 70–1 [illegible] | 0 80–2 07 |
| (vs normal) | | | | | [illegible] 33 | 9 01 |

Expressed as gm per 100 gm red blood cells
SOURCE Modified from D Y Hsia et al [118]

there are three distinct phases during the 66-day gestation period of the guinea pig embryo

1 Complete absence of liver glycogen until the fifty seventh day of gestation All enzymes concerned with synthesis can be demonstrated during this phase except amylo-(1 4 ⟶ 1 6) transglucosidase the brancher enzyme

2 Rapid accumulation of glycogen from the fifty seventh day until term at which time the content of the fetal liver is two or three times that of the maternal liver Onset of this phase coincides with the appearance of brancher activity and it has been suggested that its presence initiates the accumulation

3 Glucose 6-phosphatase activity cannot be detected in the fetal liver until birth [120] when there is a rapid depletion of the previously stored glycogen

Thus until birth the glycogenolytic enzymatic constitution of the normal fetal guinea pig liver appears to be the same as that seen in the disease under discussion It appears then that the expression of the genic abnormality in glucose 6 phosphatase deficiency disease involves a failure to provide active enzyme at birth and that the absence of the enzyme during fetal development is of no consequence Information regarding the mechanisms which determine its appearance in the normal animal at birth might have great bearing on the question of a definitive therapy for the deficiency disease The possibility that the failure of enzyme appearance is secondary to some as yet undetected hormonal abnormality cannot be denied but seems unlikely from current knowledge of biochemical genetics in bacteria and less complex organisms

colytic pathway at an increased rate By giving glucose infusions Lowe and coworkers deduced that the hypoglycemia per se was not the cause of the hyperlactic acidemia since under this circumstance the lactic acid level decreased They also found that glycogen mobilization to lactic acid from liver tissue obtained from this patient could be stimulated in vitro by glucagon but not by epinephrine and they raised the question as to whether the currently held theory of identity of glucagon and epinephrine action in regard to glycogenolysis is correct There was rapid incorporation of glucose $C^{14}$ into the liver glycogen indicating that synthesis via the UDPG glycogen transglucosylase continued despite simultaneous mobilization via phosphorylase

It would appear then that greater amounts of ingested glucose can escape sequestration in liver glycogen if glycogenolysis can be stimulated even though the products of such stimulation must be disposed of as lactate and that substantial reductions in the amount of stored glycogen can be effected with chronic stimulation and disposition via this pathway

The approach to therapy with L-thyroxine and chronic glucagon administration deserves intensive investigation not only because clinical improvement seems obtainable but also in order to clarify knowledge concerning the intermediary regulations and interrelationships in hepatic glycogen and carbohydrate metabolism

## IDIOPATHIC GENERALIZED GLYCOGENOSIS

Synonyms Pompe's disease type 2 of Cori cardiac form of generalized glycogenosis neuromuscular form of generalized glycogenosis cardiomegalia glycogenica diffusa generalized glycogenosis

It is difficult to draw a dividing line in clinical cases between the cardiac and neuromuscular forms of this disease Because no differential biochemical criterion has been found the entity will be discussed as a single one with the qualification that in certain examples the involvement of the heart predominates while in other cases the nervous system is more severely afflicted

Extensive reviews of this condition have been presented by di Sant Agnese [128 129] and by Gitzelmann [92] Ordinarily symptoms begin between the second and sixth months of life but may be present from birth Vomiting anorexia failure to grow weakness of the musculature drooling and later cyanosis and dyspnea are the most frequently observed symptoms and signs Occurrence in siblings is not uncommon

Physical findings may include

1 Appearance of imbecility not unlike that seen in cretinism or mongolian idiocy At times enlargement of the tongue occurs (Fig 6-9)

2 Hypotonia of the muscles may be extreme and lead to confusion with amyotonia congenita [130]

roentgen irradiation have all proved fruitless [122]. Several authors have claimed benefit from high protein diets of one sort or another [123], while Bulgarelli [124, 125] has been impressed with ACTH and growth hormone when combined with such diets. If a patient has biochemically proved absence of glucose 6-phosphatase, it is difficult to understand how increasing protein intake or stimulating gluconeogenesis could improve the situation in regard to maintenance of sufficient blood glucose. Glucose 6-phosphate appears to be an obligate intermediate in the conversion of protein to blood sugar by the liver. On the other hand, in debrancher deficiency limit dextrinosis, where glucose 6-phosphatase activity is adequate, there is no difficulty in releasing glucose 6-phosphate. In the latter disease such maneuvers should be highly effective on theoretical grounds for they exploit the time factor necessary for intrahepatic conversion of protein to carbohydrate and allow prolonged hepatic glucose release. These considerations reemphasize the necessity of distinguishing the two defects by direct enzymatic analysis of liver tissue.

Two other therapeutic approaches to influencing intermediary metabolism are very interesting and appear to have been met with some measure of clinical benefit. Koulischer and Pickering [126] administered L-thyroxine to three patients over a 6 week period and noted quicker rises in blood sugar after oral feedings, decline in the unfermentable fraction of the total reducing substances of the blood, improvement in the responses to glucagon and epinephrine, and a diminution of insulin sensitivity. Gitzelmann [92], employing both glucagon and L-thyroxine therapeutically, confirmed these results in one case and expressed the opinion that cases in which a good hyperglycemic response to glucagon is present before therapy are the ones most likely to respond favorably. Although no hypothesis as to the metabolic mechanisms by which these effects are achieved has been offered, the well known action of thyroxine in uncoupling oxidative phosphorylation in the Krebs cycle could be the explanation for its effect. Even though it is energetically inefficient and wasteful, increasing the rate of revolution of the Krebs cycle through uncoupling would increase the rate of disposition of carbohydrate and allow reduction in glycogen deposition.

Lowe and coworkers [127] reported a dramatic acute decrease in liver size in a patient with this disease given intensive glucagon therapy. Reenlargement of the organ occurred when therapy was stopped. When glucagon was given immediately after feedings, postprandial glycogen deposition was inhibited and there were greater increases in blood sugar than had been obtained with similar feedings without glucagon. Glucagon injections during fasting hypoglycemia did not raise the blood sugar but produced sharp rises in blood lactate. This suggests that the products of an increased glycogenolysis were being channeled through the gly-

Laboratory examinations make differentiation of this disease from glucose 6-phosphatase deficiency and the other enzyme-deficient syndromes relatively easy

1 Fasting blood sugar glucose tolerance galactose tolerance glucagon and epinephrine responses are all normal Acetonuria is not found



Fig 6-10 Roentgenogram of the chest taken at age 10 months in case of R Gitzelmann showing generalized enlargement of the globular heart with a wide overlying thymus shadow The appearance is typical of idiopathic generalized glycogenosis (*By permission of R Gitzelmann* [9 ])

2 The gross hematologic picture is normal but staining of the circulating leukocytes for glycogen will reveal massive glycogen deposition and can unmask the diagnosis

3 A globular cardiac silhouette is a common finding on roentgenographic examination of the chest (Fig 6-10)

4 Electrocardiographic changes such as depressed ST segments inverted T waves or less commonly a high spiked T wave may be encountered (Fig 6 11)

Diagnosis can be definitively established by muscle biopsy glycogen structure analysis and enzymatic assays

Histologic pathology [*129*] consists of marked myocardial infiltration with glycogen which may give a distinctive lacework appearance



Fig 6-9 *Upper* A K at age $5\frac{1}{2}$ months a case reported by R Gitzelmann of idiopathic generalized glycogenosis An older sister died at the age of $4\frac{1}{2}$ with the autopsy findings of diffuse cardiac glycogenosis with lesser involvement of liver kidneys and muscles Note resemblance to cretinism
*Lower* Same patient at age 9 month Death occurred at age $10\frac{1}{2}$ months with cardiac and circulatory insufficiency At autopsy generalized glycogenosis was found but the glycogen structural analyses and enzyme activity determinations were normal as performed by Dr G T Cori (*By permission of R Gitzelmann* [9])

3 Enlargement of the heart can usually be detected by percussion and palpation and an apical systolic murmur is not uncommon

4 Hepatosplenomegaly is only very rarely observed

5 Various neurologic deficits may be present when involvement of the nervous system is severe [*131*]

No reasonable explanation of the abnormality in glycogen metabolism in this disease is currently available although there has been speculation concerning a disturbance of hormonal regulation [92] No treatment of benefit is known and it is unlikely that any will be forthcoming until a better biochemical understanding of the pathogenesis becomes available

### DEBRANCHER DEFICIENCY LIMIT DEXTRINOSIS

Synonyms Forbes disease type 3 of Cori, limit dextrinosis

As previously mentioned it is probable that many cases described in the early literature as von Gierke s disease were examples of this defect rather than glucose 6-phosphatase deficiency glycogenosis In her last publication on the subject Cori [3] stated that 9 cases in all had been studied in her laboratory In Hers series [8] of 15 cases of glycogenosis 5 were of this variety as compared with 7 of the glucose 6-phosphatase deficiency variety Hers ventures the speculation that one of the two cases originally reported by van Creveld which developed favorably may have had amylo-1 6-glucosidase deficiency since muscle glycogen was elevated Hers perfection of the assay methods for amylo-1 6 glucosidase and a clearer understanding of the differentiating metabolic pathophysiology should make confusion of the two diseases unlikely in the future

Descriptions of the clinical manifestations of this disease are not abundant but in general they are very similar to those of glucose 6-phosphatase deficiency except that they tend to be milder and difficulties with hypoglycemia and development are less severe Glycogen deposition in the heart and skeletal muscles which does not occur in the glucose 6-phosphatase deficiency disease can give rise to a somewhat different set of clinical manifestations

In the original case described by Forbes [133] abdominal enlargement had been found at age 1 year The child had a desire for sweets and breadstuffs Acetone was noted in the fasting urine at the age of 3½ years At this time fasting blood sugars ranged between 34 and 50 mg per 100 ml serum cholesterol was 284 mg per 100 ml and there was a diminished hyperglycemic response to epinephrine The liver reached the iliac crest Subsequent development was normal and the abdominal enlargement decreased During the twelfth year the fasting blood sugars gradually reached higher levels and the serum cholesterol fell Hepatic enlargement was more prominent and marked Bromsulphalein retention was found along with an abnormal cephalin flocculation test Previously liver function tests had been normal In 1955 Recant [63] described the patient then 13 years old as appearing well A certain amount of muscle flaccidity was noted

Histologic findings in the liver biopsy at age 12 included increased fat

because of displacement of the cytoplasm by the mass of glycogen and the nuclei lying free in the center of the cell Excessive deposition of variable degrees has been noted in all muscle both smooth and striated with tongue and diaphragm frequently the most severely affected Deposits have also been observed in the reticuloendothelial system kidney cells liver cells ganglion cells cortical and medullary cells of the adrenal glands acinar ductal and islet cells of the pancreas and also in the cells of bone marrow lymph nodes and thymus Glycogen accumulation does not cause any reaction on the part of the infiltrated tissue [*132*]

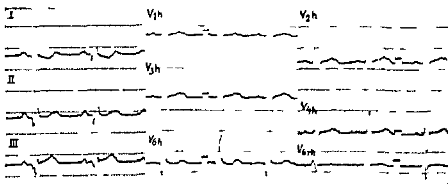



Fig 6-11 Electrocardiogram taken at age 10 months in case A K of R Gitzelmann interpreted as showing left ventricular hypertrophy and endocardial damage Such findings are not uncommon in idiopathic generalized glycogenosis (*By permission of R Gitzelmann [92]*)

In some cases infiltration with a basophilic substance interpreted as mucoprotein or mucopolysaccharide as well as excessive glycogen deposition has been noted in the skeletal muscle [*90 131*]

Biochemical examinations though documenting heart muscle glycogen concentrations as high as 10 per cent by net weight have so far failed to detect any abnormality of glycogen structure or aberration of activity of any of the enzymes known to be concerned with its metabolism [*3*] Spontaneous glycogenolysis in biopsy specimens proceeds rapidly and suggests that search for an enzymatic cause if one exists should be directed elsewhere than to the glycogenolytic systems

The central clinical problem in this disease is that of cardiac failure with tachycardia and edema In a few cases death ensues from aspiration pneumonia and progressive weakness of the muscles of respiration Death from one or another of these mechanisms within the first year is the rule

The disease is definitely familial in occurrence Gitzelmann [*92*] found 12 afflicted individuals out of a total of 21 children of seven families described in the literature The mode of inheritance appears to be through a single recessive autosomal gene

re idues which were accumulated between it and the original proximal branch point The ability to deesterify glucose 6 phosphate and release it to the blood stream as free glucose is not impaired since sufficient glucose 6-phosphatase is present This explains why gluconeogenesis from protein can defend these patients from hypoglycemia and why they demonstrate a normal hyperglucemic response to galactose and fructose infusions

Information concerning the genetics of this condition are meager Two of Cori s cases were in the same family two of Hers cases were brothers another of his ca es had a brother with glycogenosis and the parents of still another of his cases were first cousins Again inheritance through a single recessive autosomal gene seems most probable

Treatment of this di ease with its milder manifestations is less difficult than is that of glucose 6-phosphatase deficiency although the same general principles apply A more varied diet can be offered and a high protein content *may be beneficial It eems logical to avoid a diet high in fat* because of the ketogenic potentialities No reports of the effects of L-thyroxine therapy or sustained glucagon therapy in this condition have as yet appeared but one might expect that substantial glycogen mobilization from the liver via glycolysis could not be effected because of the inability to depolymerize proximal to the first branch point It is possible however that glucagon therapy might allow a greater proportion of the alimentary influx of glucose to reach the peripheral blood

Because of the probability of myocardial involvement Cori advises prohibition of strenuous exercise and Hers cautions about the use of anesthetics and cardio depre sant drugs

BRANCHER DEFICIENCY AMYLOPECTINOSIS

Synonyms Andersen s disease type 4 of Cori familial cirrhosis of the liver with storage of abnormal glycogen

This is either a very rare disease or a very difficult one to recognize only a single example has so far come to light The patient observed and studied by Andersen *[91]* was an 11½ month old boy whose brother had died at the age of 7 months with the autopsy diagnosis of von Gierke s disease The child had an enlarged liver and pleen and was icteric Fasting blood sugars ranged between 56 and 75 mg per 100 ml and there was a diminished hyperglycemic response to epinephrine The results of liver function tests were strikingly abnormal Subsequently the child developed ascites and deepening icterus and died at the age of 17 months

Autopsy revealed glycogen infiltration of liver spleen lymph nodes and intestinal mucosa Nodular cirrhosis of the liver was also present Glycogen in small amounts was present in the muscle fibers of the diaphragm but not in the kidneys

accumulation infiltration with fine granules of glycogen and a definite increase in periportal connective tissue

The functional tests which should allow presumptive clinical differentiation of this disease and the typical expected results are

1 Hypoglycemia with fasting

2 Diminished hyperglycemic response to epinephrine or glucagon depending on the amount of time since the last feeding Near normal responses are more likely in this disease because of the ability to mobilize the external chain glucose moieties if they are present as a result of a recent feeding

3 Normal hyperglucemic response to infusions of galactose and fructose provided an advanced cirrhosis is not also present

As is the case with all glycogenoses the diagnosis can be made with certainty only from the biochemical analysis of biopsy material The confirmatory biochemical findings first described by Illingworth and the Coris [31] are

1 Increased liver and muscle glycogen content with the latter in the 4 to 5 per cent range or higher

2 Abnormally structured glycogen in both liver and muscle with short external chains if obtained during fasting and an increased number of branch points (10 to 13 per cent versus 7 to 8 per cent in the normal) under any conditions Thus the molecular structure is very similar to $LD_1$ (phosphorylase) or normal glycogen hence Coris name limit dextrinosis'

3 Absence of detectable amylo-1 6-glucosidase activity with relatively normal amounts of phosphorylase and glucose 6-phosphatase

Amylo 1 6 glucosidase in contrast to glucose 6 phosphatase is normally present in numerous tissues This accounts for glycogen deposition in skeletal and cardiac muscle when the enzyme is lacking The cardiac involvement is usually not recognizable clinically Normal electrocardiographic findings must not be taken as proof that such involvement and its special hazards do not exist

Recognition of the extent and nature of the enzymatic lesion in this disease allows a better understanding of its pathophysiology Glycogen synthesis proceeds normally when glucose is in good supply and the arborized molecule is formed *With glucose deprivation or stimuli for* glycogenolysis only the external branches of the molecule can be depolymerized by phosphorylase When the first tier of branch points is reached the process stops because the specific enzyme necessary for cleavage of the 1 6 bonds is lacking With return of sufficient glucose to allow storage or withdrawal of glycogenolytic stimuli extension of the 1 4 linked external chains recommences and when they are sufficiently enlongated they are acted upon by the brancher enzyme to create a new 1 6 linked branch point This new 1 6 bond effectively traps the glucose

toms disappeared rapidly upon resting the muscle but tended to persist longer if he attempted to continue the use of the muscle The amount of work which produced pain varied from day to day and seemed to be less when he had infection There was no history of a similar disturbance in his siblings parents or progeny

Physical examination showed that he was well proportioned although he had a moderate dorsal kyphosis There were no stigmas of any of the growth dystrophies or evidence of gross intellectual deficit The size initial power and tone of all muscles were normal and no fasciculation or fibrillation could be detected nor could myoclonic phenomena be elicited Results of the neurologic examination were normal

Radiologic and hematologic findings and serum electrolytes including calcium and magnesium were all within normal limits

Findings on functional tests were as follows

1 Marked tachycardia after climbing if exercise was continued to the point of muscle weakness and ankle clonus

2 Under ischemic conditions only 10 to 20 per cent of the normal amount of work could be performed by the forearm and hand muscles

3 Maintenance of the contracted state in the exhausted muscle gave the appearance of localized swelling

4 Complete absence of electromyographic activity which had been normal at the outset of exercise in the exhausted contracted muscle

5 Following ischemic muscle work to the point of exhaustion there was a much greater reactive increase in blood flow than occurred in normal persons under the same conditions

6 Fall in concentration of lactate and pyruvate in the venous return from an ischemic muscle performing work in marked contrast to the brisk rises of both these acids under similar conditions in a normal individual

7 A normal hyperglycemic response to epinephrine but a smaller rise in blood lactate than produced in a normal person with a similar rise in blood sugar

The information from the cases reported by Pearson [*136*] and Schmid [*137*] confirms that the inability to detect an increase in blood lactate after exercise especially if performed under ischemic conditions is the best indication obtainable by functional testing that myophosphorylase deficiency is present Such a test should be performed in any investigation of muscular weakness developing after unusually short periods of exercise Pearson found that exercise tolerance in his case could be markedly improved by continuous intravenous infusion of either glucose fructose or lactate but not by galactose glycerol or fat

Histologically excessive amounts of glycogen in a diffuse granular form have been found in the muscle Unequivocal diagnosis again depends on studies of biopsy material

Structure analysis of the glycogen obtained from this case was carried out by Illingworth and Cori [134] and revealed a molecule with a structure quite similar to that of the amylopectins of plant starch Fifty per cent of the molecule could be degraded by phosphoryla e implying very long external chains There were fewer branch points and the inner chains were longer than in normal glycogen The physical properties of this abnormal molecule also resembled those of amylopectin in that it gave a purple iodine color with an absorption peak at 530 mμ while normal glycogen has a peak at 470 and corn amylopectin at 550 Normal glycogen is very soluble in cold water but this material was only sparingly soluble and reprecipitated at 0°C as does amylopectin

Cori postulated that these structural abnormalities could be explained by a decrea ed activity of the brancher enzyme amylo-(1 4 ⟶ 1 6) transglucosidase but unfortunately not enough tissue was available for the direct enzymatic assay necessary for confirmation Recant speculated that the cirrhosis might be a tissue reaction provoked by the precipitation of the relatively insoluble polysaccharide [63] Since this abnormal glycogen molecule should be subject to the usual reactions of degradation Cori suggested that its precipitation with attendant local tissue reaction made it inaccessible to phosphorylase and that this accounted for its deposition in increased amounts

More study of cases of this type of defect will have to be carried out before information concerning genetics and treatment can be obtained In earching for further examples it might be well to remember Andersen s description— Familial Cirrhosis of the Liver with Storage of Abnormal Glycogen '

## MYOPHOSPHORYLASE DEFICIENCY GLYCOGENOSIS

Synonyms McArdle s syndrome McArdle-Schmid Pearson disease type 5 of Cori

In all at least eight patients with similar clinical and laboratory findings characteristic of this disease have been observed Tissue from two of them has been subjected to exhaustive biochemical studies [135–137] which have elucidated a single enzyme deficiency lack of muscle phosphorylase as the cause of the condition

Although McArdle [138] did not have the advantage of biochemical studies of excised tissue and interpreted the results of his physiologic studies as indicating a lesion of muscle glyceraldehyde phosphate dehydrogenase it seems almost certain that his patient had this disease His clinical description is the most complete presently to be found in the literature and will be used to characterize the clinical features

The patient was a 30 year old man who for as long as he could remember had experienced first pain and then weakness and stiffne s with variable periods of exercise of any muscle including the masseter Symp

intact chain of associated enzymes along a given metabolic pathway strongly suggests that the defect is a result of a single genic determination There is no difficult in understanding the mechanisms by which characteristic clinical symptoms and signs are produced during exercise since there is a biochemical lesion which prohibits the release of reserve fuel The anomalous fall in lactate with muscle work probably indicates that it is being used to make up the fuel deficit at the same time that its production is being curtailed

Beyond advice to avoid exercise to the extent of producing symptoms there seems little to offer these patients therapeutically Perhaps the constant use of glucose lozenges during periods of necessary activity might prolong the duration of the symptom free phase Avoidance of tight garters cuffs collars and girdles would seem to be indicated also

## HEPATOPHOSPHORYLASE DEFICIENCY GLYCOGENOSIS

Synonyms Hers disease type 6 of Cori

In his investigation of 15 cases of glycogenosis Hers found three apparently unrelated persons with an approximately 75 per cent reduction in assayable liver phosphorylase [8] In two of the cases the liver glycogen content was over 12 per cent in the third case despite a glycogen content of only 2 2 per cent assurance is given that the clinical and histopathologic criteria for a glycogenosis were fulfilled Assay of glucose 6-phosphatase amylo 1 6 glucosidase in liver and muscle and muscle phosphorylase gave near normal values Muscle glycogen content was normal

No clinical description of the cases has as yet been provided but it might be guessed that they resemble patients with glucose 6 phosphatase deficiency disease or debrancher deficiency limit dextrinosis with fasting hypoglycemia and subnormal hyperglycemic responses to glucagon As in limit dextrinosis the galactose or fructose infusion test results in a marked hyperglucemia

The intrinsic nature of this phosphorylase defect had not been completely elucidated at the time of the report but the presence of some phosphorylase activity apparently led the author to suspect a primary defect in either the dephosphophosphorylase kinase or the phosphorylase-phosphatase systems even though additions of ATP $Mg^{++}$ and adenosine 3 5 monophosphate failed to increase activity It was also pointed out that since phosphorylase is the rate limiting step in glycogenolysis and since its activity in the phosphorolytic direction is from 2 5 to 5 5 weaker than in the synthetic direction a 75 per cent reduction of activity (assayed under optimal conditions in the synthetic direction) would account for the excessive glycogen deposition The normality of the glycogen content and phosphorylase activity found in the muscles is another indication among other evidences that myophosphorylase

Such biochemical studies on excised muscle from patients with this disease have shown the following

1 Increased deposition of glycogen (2 5 to 4 1 per cent versus 0 2 to 0 9 per cent normally)

2 Inability of homogenates of biopsy tissue to form lactate in vitro from endogenous glycogen or added normal human glycogen, but an intact glycolytic capacity as indicated by normal lactate production after addition of glucose 1 phosphate

3 Normally structured glycogen by analytic enzymatic degradation [59]

4 Essentially undetectable phosphorylase *a* and *b* activity despite additions of AMP or pyridoxal 5-phosphate Additions of crystalline phosphorylase to homogenate show no loss in activity

5 Adequate activity of the enzymes concerned with phosphorylase *a* and *b* interconversion, i e, PR enzyme and phosphorylase kinase The other enzymes concerned with glycogen synthesis and breakdown can also be detected as functionally adequate i e phosphoglucomutase amylo-1 6-glucosidase UDPG glycogen transglucosylase and UDPG pyrophosphorylase [5o 59 187]

Since excessive glycogen deposition occurs in the muscles in this disease and there is absence of phosphorylase synthesis must occur via the UDPG glycogen transglucosylase pathway and this fact has been previously presented as part of the evidence for partition of glycogen synthesis and breakdown Why greater accumulation of glycogen such as is seen in idiopathic generalized glycogenosis does not occur in muscles without phosphorylase remains a mystery Whether cardiac muscle is also involved in myophosphorylase deficiency remains to be answered when studies of autopsy material can be made Since cardiac muscle habitually uses substrates other than glucose as the major fuels of its energy production involvement with this defect might not be detectable or incapacitating No interference with mechanical action due to the moderate glycogen deposition is detectable in the rested skeletal muscle and a similar degree of glycogenosis of cardiac muscle need not interfere with its contractile capacity

It is clear from the normal results obtained with glucagon tests that hepatic phosphorylase activity is intact in this disease This fact is not surprising when it is recalled that the two enzyme proteins are immunologically distinct and have different mechanisms of activation and deactivation It seems highly probable therefore that there is a separate gene for each of the two phosphorylases if this disease is genetically determined Insufficient information is available concerning sibling occurrence sex distribution consanguinity of parents and familial occurrence to reach a conclusion regarding the mode of inheritance of this defect However the sharp specific absolute absence of a single enzyme in an otherwise

inten e interest to biochemists since the time of Claude Bernard and have been worked out with considerable clarity

2 Humoral and other regulatory factors can influence the ebb and flow of glycogen metabolism The mechanisms by which such agents act are in some cases obscure and complex but much information concerning their actions is already available Certain tis ues appear to have special ized systems in regard to the glycogen cycle This fact makes it necessary to characterize the glycogen metabolism of any given tissue independently

3 Clinical examples of excessive glycogen deposition in several or all organs and ti sues have been observed Five of the six currently recognized diseases appear to be biochemical ' lesions due to a genetically determined absence or deficiency of a single enzyme concerned with the degradation or structural modification of stored glycogen In each of these syndromes the mode of transmission seems to be as a simple auto-somal recessive gene A form of generalized glycogenosis is known in which as yet no enzymatic or biochemical lesion has been elucidated

4 The steps of the glycogen cycle and the enzymes involved appear in schematic form in Fig 6-12 The point of blockage and the genetically determined enzyme deficiency are indicated along with the suggested clinical name for each syndrome

5 Consideration of the position of each lesion in the framework of the knowledge concerning the steps of the glycogen cycle has made possible understanding of many of the clinical findings as well as proper interpretation of functional diagnostic tests Definitive diagnosis cannot be made from clinical and functional test information with ab olute certainty Direct investigation of the activities of the various pertinent enzymes in the involved tissues must be made in order to characterize unequivocally any given example of a glycogen deposition di ease

6 At the present therapy in these di eases is defensive and supportive Better understanding of the biochemistry involved has allowed employ ment of logical measures which in many instances are gratifyingly effective

7 It now appears that a human disease state has been found for the isolated deficiency of every currently de cribed enzyme whose absence could conceivably lead to excessive glycogen deposition Nevertheless as astute clinicians continue to scrutinize their cases they will provide undoubtedly problems questions and clues along with tissue for study by their biochemist allies and diseases of greater subtlety and complexity will be *recognized and understood*

## BIBLIOGRAPHY

1 Cori G T and Cori C F Glucose 6-pho phatase of the liver in glycogen storage di ease J Biol Chem 199 661 1952

and hepatic phosphoryla.e are different enzyme proteins which may have separate genic determinants

Until more is known concerning the e ential nature of this defect and more cases are recognized and studied as to genealogy no statement can be made concerning its mode of inheritance or methods of therapy

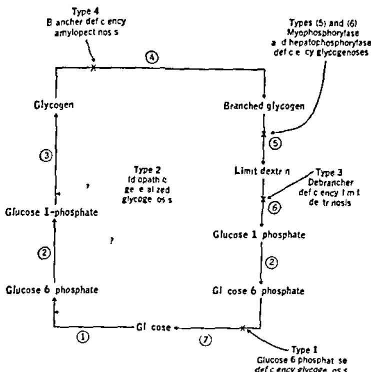



Fig 6-1? The glycogen cycle ( chet stic ) an l it s of genetically determ ne l enzyme lesions causing exce ive glycogen dep ition

## SUMMARY

1 Glycogen a polydisper e polysaccharide of high molecular weight present in almost all ti ues of the animal body occupies a role in the economy of metabolism as a storage carbohydrate similar to the role of the starches and amylopectins in the plant kingdom The enzymatic steps involved in its synthe is and degradation have been the subject of

26 Vuylsteke C A and deDuve C Augmentation de l effet du glucagon por un facteur présent dans les préparations d insuline spécificité du phénomène J Physiol 47 307 1955
27 Vuylsteke C A and deDuve C The assay of glucagon on isolated liver slices Arch internat pharmacodyn 111 437 1957
28 Sutherland E W and Cori C F Effect of hyperglycemic-glycogenolytic factor and epinephrine on liver phosphorylase J Biol Chem 188 531 1951
29 Cori G T and Larner J Action of amylo 1 6-glucosidase and phosphorylase of glycogen and amylopectin J Biol Chem 188 17 1951
30 Cori G T Enzymic structure analysis and molecular weight determination of polysaccharides Makromoleculare Chemie 20 169 1956
31 Illingworth B Cori G T and Cori C F Amylo-1 6-glucosidase in muscle tissue in generalized glycogen storage disease J Biol Chem 218 123 1956
32 Cardini C E Paladini A C Caputto R Leloir L F and Trucco R E The isolation of the coenzyme of phosphoglucomutase Arch Biochem 22 87 1949
33 Najjar V A The isolation and properties of phosphoglucomutase J Biol Chem 176 81 1948
34 Ginsburg V and Neufeld E F Inhibition of phosphoglucomutase by galactose 1 phosphate A possible factor in galactose toxicity Abstract Am Chem Soc Meeting 27C 1957
35 Sidbury J B Jr The enzymatic lesions in galactosemia J Clin Invest 36 929 1957
36 deDuve C Berthet J Hers H G and Dupret L Le systeme hexose phosphatasique existence d une glucose 6-phosphatase spécifique dans le foie Bull Soc chim biol 31 1242 1949
37 Hers H G Berthet J Berthet L and deDuve C Le systeme hexose phosphatasique III Localisation intracellulaire des ferments par centrifugation fractionnée Bull Soc chim biol 33 21 1951
38 Hers H G Les système hexosephosphatasique répartition de l activité glucose 6-phosphatasique dans les tissus Bull Soc chim biol 32 20 1950
39 Beaufay H and deDuve C Le système hexose phosphatasique IV Specificite de la glucose 6-phosphatase Bull Soc chim biol 36 15 5 1954
40 Crane R K The substrate specificity of liver glucose-6-phosphatase Biochim et biophys acta 17 443 1955
41 Langdon R G and Weakley D R The influence of hormonal factors and of diet upon hepatic glucose 6-phosphatase activity J Biol Chem 214 167 1955
42 Ashmore J Cahill G F and Hastings A B Inhibition of glucose 6-phosphatase by hypoglycemic sulfonylureas Metabolism 5 774 1956
43 Freedland R A and Harper A E Metabolic adaptations in higher animals III Quantitative study of dietary factors causing response in liver glucose-6-phosphatase J Biol Chem 233 1 1958
44 Segal H L and Washko M E Studies of liver glucose 6-phosphatase III Solubilization and properties of the enzyme from normal and diabetic rats J Biol Chem 234 1937 1959
45 Park C R Reinwein D Henderson M J Cadenas R and Morgan H E The action of insulin on the transport of glucose through the cell membrane Am J Med 26 674 1959
46 Crane R K and Krane S M Studies on the mechanism of the intestinal active transport of sugars Biochim et biophys acta 31 397 1959
47 Field R A Unpublished observations
48 Slein M W Cori G T and Cori C F A comparative study of hexokinase from yeast and animal tissues J Biol Chem 186 763 1950
49 Crane R K and Sols A The association of hexokinase with particulate fractions of brain and other tissue homogenate J Biol Chem 203 273 1953
50 Cori C T and Slein M W Gluco and fructokinase in mammalian tissu Fed Proc 6 24 1947
51 Leloir L F The enzymatic transformation of uridine diphosphate glucose into a galactose derivative Arch Biochem & Biophys 33 186 1951

2 Ochoa S Gerty T Cori Biochemist Science 128 16 1958
3 Cori G T Biochemical aspects of glycogen deposition diseases Mod Problems Paediat 3 344 1957
4 Jasmin R and Johnson W Inhibition of glucose 6-phosphatase by thiadiazoles J Am Pharm A (Scient Ed) 48 113 1959
5 Cori G T Glycogen structure and enzyme deficiencies in glycogen storage disease Harvey Lect 48 145 1950-53
6 Cori C F Schmidt G and Cori G T The synthesis of a polysaccharide from glucose 1 phosphate in muscle extract Science, 89 464 1939
7 Cori C F in *Carbohydrate Metabolism A Symposium on the Clinical and Biochemical Aspects of Carbohydrate Utilization in Health and Disease* edited by V A Najjar Johns Hopkins Press Baltimore 1952
8 Hers H G Études enzymatiques sur fragments hépatiques application à la classification des glycogénoses Rev internat d hépatol 9 35 1959
9 Leloir L F and Cardini C E Biosynthesis of glycogen from uridine diphosphate glucose J Am Chem Soc 79 6340 1957
10 Robbins P W Traut R R and Lipmann F Glycogen synthesis from glucose glucose 6-phosphate and uridine diphosphate glucose in muscle preparations Proc Nat Acad Sci (U S) 45 6 1959
11 Leloir L F Olavarria J M Goldemberg S H and Carminotti H Biosynthesis of glycogen from uridine diphosphate glucose Arch Biochem & Biophys 81 508 1959
12 Sutherland E W Jr Hormonal activation of phosphorylase in *Enzymes Units of Biological Structure and Function* edited by O H Gaebler p 536 Academic Press Inc New York 1956
13 Rall T W Sutherland E W and Berthet J The relationship of epinephrine and glucagon to liver phosphorylase IV Effect of epinephrine and glucagon on the reactivation of phosphorylase in liver homogenates J Biol Chem 224 463 1957
14 Rall T W and Sutherland E W Formation of a cyclic adenine ribonucleotide by tissue particles J Biol Chem 232 1065 1958
15 Cori G T and Illingworth B The effect of epinephrine and other glycogenolytic agents on the phosphorylase a content of muscle Biochim et biophys acta 21 105 1956
16 Cori C F Cori G T and Green A A Crystalline muscle phosphorylase III Kinetics J Biol Chem 151 39 1943
17 Green A A and Cori G T Crystalline muscle phosphorylase I Preparation properties and molecular weight J Biol Chem 151 21 1943
18 *Baranowski T Illingworth B Brown D H and Cori C F The isolation of* pyridoxal 5-phosphate from crystalline muscle phosphorylase Biochim et biophys acta 25 16 1957
19 Keller P J and Cori G T Purification and properties of the phosphorylase rupturing enzyme J Biol Chem 214 127 1955
20 Krebs E G Kent A B Graves D J and Fischer E H The activation and inactivation of muscle phosphorylase Proc Internat Symposium Enzyme Chem Tokyo and Kyoto p 41 1957
21 Krebs E G Kent A B and Fischer E H The muscle phosphorylase b kinase reaction J Biol Chem 231 73 1958
22 Henion W F and Sutherland E W Immunological differences of phosphorylases J Biol Chem 224 477 1957
23 Sutherland E W and Wosilait W D The relationship of epinephrine and glucagon to liver phosphorylase I Liver phosphorylase preparation and properties J Biol Chem 218 459 1956
24 Rall T W Sutherland E W and Wosilait W D The relationship of epinephrine and glucagon to liver phosphorylase III Reactivation of liver phosphorylase in slices and in extracts J Biol Chem 218 483 1956
25 Hastings A B Ashmore J and Cahill G F Jr Intracellular ionic environment and enzyme activities carbohydrate metabolism in liver Arch Biochem & Biophys 65 78 1956

77 Vasella F Die Glukagonbelastungsprobe im gesunden Kind Helvet paediat acta 12 331 1957
78 Bencasme S A and Frei J Relation of glucagon to alpha cells of the pancreas Proc Soc Exper Biol & Med 91 589 1956
79 Staub A Sinn L and Behrens O Purification and crystallization of glucagon J Biol Chem 214 619 1954
79a Bromer W W Sinn L G Staub A and Behrens O K The amino acid sequence of glucagon Diabetes 6 234 1957
80 Goldner M G Jauregui R H and Weisenfeld S Disappearance of HGF from insulin after liver perfusion Am J Physiol 179 25 1954
81 Narahara H T and Williams R H Effect of protein a1 led in vitro upon insulin degradation and glucose uptake by muscle J Biol Chem 233 1034 1958
82 Hill R L and Smith E L Complete hydrolysis of glucagon by leucine amino peptidase Biochim et biophys acta 31 257 1959
83 Sutherland E W Glucagon and epinephrine in *Diabetes* edited by R H Williams chap 15 p 184 Hoeber New York 1960
84 Kirtley W R Waife S O Helmer O M and Peck F B Effect of purified glucagon (hyperglycemic glycogenolytic factor HGF) on carbohydrate and corticoid metabolism in normal and diabetic subjects Diabetes 2 345 1953
85 Williams R H Thyroid in *A Textbook of Endocrinology* pp 83 114 Saunders Philadelphia 1950
86 Hoch F L and Lipmann F The uncoupling of respiration and phosphorylation by thyroid hormones Proc Nat Acad Sc (U S) 40 909 1954
87 Toreson W E Glycogen infiltration (so-called hydropic degeneration) in the pancreas in human and experimental diabetes mellitus Am J Path 27 327 1951
88 Cogan D G Personal communication
89 Chaptal J Jean R Campo C Loubatières R Alram D and Bennet H Étude sur les polycories glycogéniques hépatiques à propos d quatre ob rvations Arch franç paediat 12 454 1955
90 Zellweger H Glykogenspeicherkrankheiten Deutsche med Wchnschr 81 1907 1956
91 Andersen D H Familiar cirrhosis of the liver with storage of abnormal glycogen Lab Invest 5 11 1956
92 Gitzelmann R Glukagonprobleme bei den Glykogenspeicherkrankheiten Helvet paediat acta 12 4 5 1957
93 Lereboullet I Sur un cas de cirrhose biliare anticterique Bull Soc pediat Paris 89 1901
94 Wagner R and Parnas J K Ueber eine eigenartige Storung des Kohlenhydratstoffwechsels und ihre Beziehungen zum Diabetes mellitus Eine klinisch experimentelle Studie II Ztschr ges exper Med 25 361 1921
95 von Pfaundler M Hepatischer Infantilismus? Ztschr Kinderh 41 78 1926
96 Snapper I and van Creveld S Un cas d hypoglycémie avec acéton mie chez un enfant Bull et mém Soc méd hôp Paris 52 1315 1928
97 von Gierke E Hepato nephromegal a glykogenia (Glykogen peich krankheit den Leber und Nieren) Beitr path Anat 82 497 1929
98 Bischoff G Zum klinischen Bild der Glykogen Speicherungskrankheit (Glykogenose) Ztschr Kinderh 52 722 193
99 Schonheimer R Über eine eigenartige Storung des Kohlenhydrat Stoffwechsels Ztschr physiol chem 182 148 1929
100 Kimmelstiel P Über Glykogenose Beitr path Anat 91 1 1933
101 Wagner R Die Speicherkrankheiten (Thesaurismosen) Erg bn inn Med u Kinderh 53 586 1937
102 van Creveld S Glycogen disease Medicine 18 1 1939
103 von Creveld S Glycogen disease Arch Dis Childhood 27 113 195 1953
104 Zakon S J Oyamada A and Rosenthal I H Eruptive xanthoma and hyperlipemia in glycogen storage disease (von Gierke s disease) A M A Arch Dermat & Syph 67 146 1953
105 Schulman J L and Saturen P Glycogen storage disease of the liver I Clinical studies during the early neonatal period Pediatrics 14 632 1954

52 Siu I M L and Wood H G Conversion of galactose and glucose to liver glycogen in vivo J Biol Chem 234 2?3 1959
53 Everett M R in *Medical Biochemistry* 2d ed chap 1 p 307 Hoeber New York 1946
54 Hers H G The conversion of fructose 1 $C^{14}$ and sorbitol 1 $C^{14}$ to liver and muscle glycogen in the rat J Biol Chem 214 373 1955
55 Larner J and Villar Palasi C Enzymes in a glycogen storage myopathy Proc Nat Acad Sc (U S) 45 1234 1959
56 Villar Palasi C and Larner J A uridine coenzyme linked pathway of glycogen synthesis in muscle Biochim et biophys acta 30 449 1958
57 *Hauk R and Brown D H Preparation and properties of uridinediphosphoglucose-glycogen transferase from rabbit muscl* Biochim et biophys acta 33 556 1959
58 Hauk R Illingworth B Brown D H and Cori C F Enzymes of glycogen synthesis in glycogen-deposition disease Biochim et biophys acta 33 554 1959
59 Mommaerts W F H M Illingworth B Pearson C M Guillory R J and Seraydarian K A functional disorder of muscle associated with the absence of phosphorylase Proc Nat Acad Sc (U S) 45 791 1959
60 Larner J The action of branching enzymes in outer chains of glycogen J Biol Chem 202 491 1953
61 Paladini A C Caputto R Leloir L F Trucco R E and Cardini C E *The enzymatic synthesis of glucose 1 6-diphosphate Arch Biochem 23 55 1949*
62 Roche J Phosphatases in *The Enzymes* edited by J B Sumner and K Myrbäch part 1 vol 1 p 473 Academic Press Inc New York 1950
63 Recant L Recent developments in the field of glycogen metabolism and the diseases of glycogen storage Am J Med 19 610 1955
64 Baker N Chaikoff I L and Schusdek A Effect of fructose on lipogenesis from lactate and acetate in diabetic liver J Biochem 194 435 1952
65 Helmreich E and Cori C F Studies of tissue permeability II The distribution of pentoses between plasma and muscle J Biol Chem 224 663 1957
66 Gemmill C L The effect of insulin on the glycogen content of isolated muscles Bull Johns Hopkins Hosp 66 23 1940
67 Shaw W N and Stadie W C Two identical Embden Meyerhof enzyme systems in normal rat diaphragms in cytological locations and response to insulin J Biol Chem 234 2491 1959
68 Berthet J Jacques P Hers H G and deDuve C *Influence de l insuline et du glucagon sur la slynthèse du glycogène hépatique Biochim et biophys* acta 20 190 1956
69 Renold A E Hastings A B Nesbett F B and Ashmore J Studies on carbohydrate metabolism in rat liver slices IV Biochemical sequence of events after insulin administration J Biochem 213 135 1955
70 Dorfman R I Ross E and Shipley R A The assay of adrenal cortical material by means of a glycogen test in the adrenalectomized mouse Endocrinology 38 178 1946
71 Rosen F Roberts N R and Nichol C A Glucocorticosteroids and transaminase activity *I Increased activity of glutamic pyruvic transaminase in four* conditions associated with gluconeogenesis J Biol Chem 234 476 1959
72 Park C R in *A Symposium on the Clinical and Biochemical Aspects of Carbohydrate Utilization in Health and Disease* edited by V A Najjar chap 4 p 43 Johns Hopkins Press Baltimore 1952
73 Price W H Cori C F and Colowick S P The effect of anterior pituitary extract and of insulin on the hexokinase reaction J Biol Chem 160 633 1945
74 Colowick S P Cori G T and Slein M W The effect of adrenal cortex and anterior pituitary extracts and insulin on the hexokinase reaction J Biol Chem *168 583 1947*
75 Haynes R C Jr The activation of adrenal phosphorylase by the adrenocorticotropic hormone J Biol Chem 233 1 ?0 1958
76 Cornblath M Reactivation of rabbit liver phosphorylase by epinephrine glucagon and epinephrine Am J Physiol 183 240 1955

133 Forbes G B Glycogen di ease Report of a case with abnormal glycogen storage structure in liver and skeletal mu cle J Pediat 42 645 1953
134 Illingworth B and Cori G T Structure of glycogens and amylopectins III Normal and abnormal human glycogen J Biol Chem 199 653 1952
135 Schmid R Robbins P W and Taut R R Glycogen synthesis in muscle lacking pho phorylase Proc Nat Acad Sc (U S) 45 1236 1959
136 Pearson C M Rimer D G and Mommaerts W F N M Defect in muscle phosphorylase a newly defined human disease Clin Res 7 298 1959
137 Schmid R and Mahler R Syndrome of muscular dystrophy with myoglobinuria demonstration of a glycogenolytic defect in muscle J Clin Invest 38 1040 1959
138 McArdle B Myopathy due to a defect in muscle glycogen breakdown Clin Sc 10 13 1951

106 McGovern J Personal communication
107 Lanconi G and Bickel H Die chronische Aminoaciduríe (Aminosäurediabetes oder nephrotisch glukosurischer Zwergwuchs) bei der Glykogenose und der Cystinkrankheit Helvet paediat acta 4 359 1949
108 Ullstrom R A Ziegler M R Doeden D and McQuarrie T Metabolic and clinical effects of corticotropin (ACTH) on essential glycogenosis (von Gierke's disease) Metabolism 1 291 1952
109 Wagner R Meyerriecks N and Sparaco R Glycogen storage disease (phosphorylated intermediates in the peripheral blood) J Pediat 53 683 1958
110 Carson M J and Koch R Clinical studies with glucagon in children J Pediat 47 161 1955
111 Schwartz R Ashmore J and Renold A E Galactose tolerance in glycogen storage disease Pediatrics 19 585 1957
112 Hers H G and Malbrain H Helvet paediat acta (in press)
113 Illingworth B and Cori G T Structure of glycogens and amylopectins III Normal and abnormal human glycogen J Biochem 199 653 1952
114 Harris R C and Olmo C Liver with kidney glucose 6-phosphatase activity in children with normal and diseased organs J Clin Invest 33 1 01 1954
115 Chiquoine A D Distribution of glucose 6-phosphatase in liver and kidney of mouse J Histochem 1 429 1953
116 Andersen D H in A Symposium on the Clinical and Biochemical Aspects of Carbohydrate Utilization in Health and Disease edited by V A Najjar Johns Hopkins Press Baltimore 1952
117 Neel J V and Schull W J Human Heredity p 163 University of Chicago Press Chicago 1954
118 Hsia D Y Y and Kot L G Detection of heterozygous carriers in glycogen storage disease of the liver Nature London 183 1331 1959
119 Nemeth A M Insull W Jr and Flexner S B Glycogenesis in the liver of the fetal guinea pig J Biol Chem 208 765 1954
120 Nemeth A M Glucose 6-phosphate in the liver of the fetal guinea pig J Biol Chem 208 773 1954
121 Lowrey G H and Wilson J L Observations on the treatment of a case of glycogen storage disease J Pediat 35 702 1949
122 Bridge E M and Holt L E Jr Glycogen storage disease Observations on the pathologic physiology of two cases of the hepatic form of the disease J Pediat 27 299 1945
123 Traisman A S and Traisman H S Glycogen storage disease of the liver in siblings J Pediat 42 651 1953
124 Bulgarelli R Sull etiopatogenesi e della malattia glicogenica ricerche varie dopo somministrazione di ACTH e di glucagone Minerva pediat 7 838 1955
125 Bulgarelli R Sui presunti rapporti tra ormone somatropo prepotuitario (STH) e glucagone Prime ricerche de somministrazione di STH nella malattia glicogenica Minerva pediat 8 37 1956
126 Koulischer N and Pickering D E Glycogen-storage disease study on effect of sodium l thyroxine and glucagon A M A Am J Dis Child 91 103 1956
127 Lowe C U Sokal J E Doray B H and Sirecione L J Biochemical studies and specific therapy in hepatic glycogen storage disease J Clin Invest 38 1021 1959
128 di Sant Agnese P A Andersen D H and Mason H Glycogen storage disease of the heart II Critical review of the literature Pediatrics 6 607 1950
129 di Sant Agnese P A Diseases of glycogen storage with special reference to the cardiac type of generalized glycogenosis Ann New York Acad Sc 72 439 1959
130 Clement D H and Godman G C Glycogen disease resembling mongolism cretinism and amyotonia congenita J Pediat 36 11 1950
131 Sellberg W Die Glykogenose des Säuglings unter dem Bilde einer tödlich verlaufenden cerebrospinalen Erkrankung Ztschr Kinderh 72 306 1953
132 van Creveld S Glycogen disease Postgrad Med 14 312 1953

milk ingestion develop vomiting and diarrhea which are usually followed by dehydration The subsequent poor caloric intake and gastrointestinal losses contribute to impaired nutrition and growth Jaundice is a common occurrence and reflects the parenchymal liver involvement that often accompanies the disease Initially the liver shows fatty infiltration which

TABLE 7 1 CLINICAL AND LABORATORY FINDINGS IN GALACTOSEMIA

| *Clinical Features* | *Laboratory Findings* |
|---|---|
| 1 Nutritional failure | 1 Elevated blood galactose |
| 2 Hepatosplenomegaly cirrhosis | 2 Abnormal galactose tolerance |
| 3 Cataracts | 3 Deficient erythrocyte galactose 1 phosphate uridyl transferase |
| 4 Mental retardation | 4 Urine galactose amino acids albumin |

readily proceeds to cirrhosis if the milk ingestion persists In some cases the disease is fulminating and death may occur in the neonatal period or soon thereafter If the clinical manifestations extend beyond 4 to 8 weeks one can usually detect evidence of cataract formation followed subsequently by mental retardation These infants are usually reluctant to eat and may show lethargy and hypotonia Depression of blood glucose levels during milk ingestion is not unusual and symptoms typical of hypoglycemia may occur There may exist therefore an elevated blood galactose level together with a low blood glucose This may also occur during a galactose tolerance test (Fig 7 1) The experimental observations of Fox [4] suggest that the hypoglucosemia may be due to stimulation of insulin release from the pancreas by the elevated blood galactose

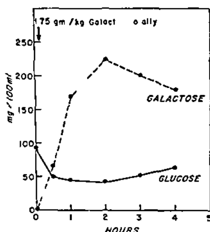



Fig 7 1 Effect of an oral galactose tolerance test on blood glucose and galactose levels in an 8-year-old patient with galactosemia

It should be emphasized that in addition to cases of galactosemia with the classic features as described above there are other patients with less pronounced manifestations in whom disturbances of growth liver function or the central nervous system may not attract attention for weeks or months Frequently these patients are ones who have shown milk intolerance in the early days of life Presumably because of reduced milk intake and the use of milk substitutes they have not developed the severe clinical manifestations It is readily apparent how the diagnosis may be missed in such patients on clinical grounds alone Patients

## Chapter 7

# Galactosemia*

*Kurt J. Isselbacher*

The disease galactosemia is now well recognized as a congenital and hereditary error in the metabolism of galactose. Patients afflicted with this disorder have an impairment in their ability to utilize ingested galactose and to convert it to glucose or energy. The clinical manifestations are directly related to galactose administration and the disease may be fatal if galactose ingestion continues. In contrast, upon removal of the offending carbohydrates from the diet, most of the symptoms regress or even disappear completely. More than forty cases have been reported and are known to exist at the present time [1-3] and many more unreported cases have been discovered in the course of investigation of this disease.

It is the purpose of this chapter to discuss the clinical aspects of the disease and current methods available for its diagnosis and treatment. The biochemical reactions important in galactose metabolism and specifically the basic enzymatic defect in galactosemia are reviewed in detail. The current concepts of the genetic mode of transmission of the disease and the techniques available for the detection of the heterozygous state are also described.

### CLINICAL ASPECTS

The symptoms which occur in galactosemia usually manifest themselves shortly after birth and are known to be causally related to the ingestion of galactose as found in the lactose of milk. The main clinical features in the classic cases include the triad of cataract formation, hepatosplenomegaly, and mental retardation (Table 7 1). The affected infants usually appear normal at birth but after some days or weeks of

* This work was supported in part by grant from the National Institutes of Health (A 1332).

completely [2] The only major finding in which there u ually is no improvement is the mental retardation It has been ob erved by most authors that the central nervous system damage may not improve de pite the mo t rigid adherence to a galactose-free diet It is for this rea on that early diagnosis and prompt institution of therapy are o important

Galactose free diets can be in tituted in infancy by re orting to any number of commercially available milk substitutes such as Nutramigen Dextri Maltose or soy bean preparations It has been amply demon strated that these diets are compatible with normal development and growth even though as Holzel et al [11] point out they may still con tain traces of galactose Most of the patients whom the author has main tained on these commercial preparations have done well In none have mental retardation cataract formation liver di ease or any symptoms occurred as a result of these diets The author therefore does not share the view of Holzel et al that the traces of galactose in these commercial milk substitutes are harmful and that more strict artificial diets hould be instituted On the contrary there is the consideration that the gastro-intestinal disturbances that accompany the lactose-free diet of Holzel Komrower and Schwarz [12] may occasionally be a drawback in the very young infant

Although Platt [13] feels that galactose ingestion may be of special benefit and that galactose is poorly synthe ized in the body there is at present no definite clinical evidence that central nervous system function or somatic development in general is impaired by the prolonged use of galactose free diets There is ample evidence as indicated below that patients with galactosemia possess the enzyme uridine dipho phate galactose 4 epimera e which allows for endogenous synthesis of galacto e from glucose and hence permits galactolipid formation in the central nervous system [14 15] and elsewhere in the absence of dietary galactose

Gal icto emia is now known to be due to the congenital absence of a specific enzyme galactose 1 phosphate uridyl tran ferase [16 17] which accounts for the imp ured galactose metaboli m in this disease Neverthe le s it has been frequently ob erved that patients with g ilactosemia may in later years of life ingest varying quantities of galactose without ex periencing any significant side effects [1 3] This phenomenon is probably rel ited to two factors (1) an actual improvement in the g ilactose utiliza tion in these patients with increasing age and (2) the fact that in later years the ratio of ingested galacto e to body weight or urface area i far smaller than in the neonat il period when the symptoms are so pronounced

The improvement in g ilacto e tolerance with age at fir t eems para doxical since even the adult has been shown to have a per istence of the enzymatic defect One explanation is that there is an additional enzyme permitting galacto e utilization (see below UDI galacto e pyrophos phoryl e) which in the normal person is quite deficient at birth but

in mental institutions who have recently been discovered by means of the red cell enzyme test (see below) [9] to have galactosemia probably fall into this group

*The earliest clue to the diagnosis of galactosemia is usually the finding* of a reducing sugar in the urine which when analyzed (see below) is identified as galactose It is important to emphasize that the galactosuria may be inconstant for it is obviously dependent on galactose or lactose ingestion and this may be minimal if the patient has been vomiting Errors of diagnosis have often been made when the patient was placed on parenteral fluid therapy upon admission to the hospital and the *reducing sugar shown by initial urinalysis was mistakenly assumed to be* due to the intravenous glucose

In addition to galactosuria these patients may have variable degrees of albuminuria and aminoaciduria [6–8] The amino acid pattern has been similar in most cases There is a predominance of the neutral simple aliphatic chain type i e serine glycine alanine threonine glutamine and valine In addition small quantities of phenylalanine, lysine cystine *glutamic acid methyl histidine tyrosine and aminoisobutyric acids* have been detected [6 7] It has been shown by Komrower [9] as well as by Cusworth Dent and Flynn [10] that this aminoaciduria is primarily renal in origin It occurs only in relation to galactose ingestion and galactosuria and is not observed in patients on galactose-free diets In a study by Cusworth et al [10] the urinary amino acid excretion was evident for as long as 7 days after the cessation of galactosuria It is assumed that galactose or one of its metabolites interferes with amino-acid reabsorption by the renal tubule The fact that the aminoaciduria occurs only after several days of galactosuria [10] and persists for some days after galactose has disappeared from the urine is perhaps more in favor of the exertion of an inhibitory effect on the renal tubule by a metabolite of galactose Galactose 1 phosphate which is known to accumulate in the tissues of galactosemic patients (see below) may be the metabolite which inhibits the renal tubular reabsorption of amino acids

## NUTRITIONAL THERAPY AND SUBSEQUENT COURSE

If significant milk ingestion persists in patients with galactosemia death usually results On the other hand if the diagnosis is made before the disease is too far advanced one of the striking features is that all the symptoms and signs may regress and even disappear completely if the patients are given galactose-free diets Nausea vomiting and diarrhea cease and there is rapid weight gain The liver and spleen return to normal size Cirrhosis if present has been known to disappear completely [1] The urinary abnormalities mentioned above likewise promptly cease Cataracts if present often regress and in some instances disappear

phogalactose (UDP galactose) while at the same time glucose 1 phosphate is liberated from the original nucleotide

**Galactose 1 phosphate + UDP glucose ⇌ UDP galactose + glucose 1 phosphate (2)**

The enzyme has been variously referred to as Gal 1 P uridyl transferase P Gal transferase or Gal 1 P transuridylase This enzyme is of prime importance for the utilization of galactose 1 phosphate and its incorporation into UDP galactose

It should be noted that another reaction (5) (see below) can also serve to incorporate galactose 1 phosphate into UDP galactose but does so to a lesser extent UDP galactose is necessary for the synthesis of galactose derivatives such as lactose galactolipids and galactose-containing polysaccharides (e g chondroitin sulfate) That Gal 1 P uridyl transferase represents the main pathway in galactose utilization will be evident from the fact that this enzyme as described below is blocked or deficient in galactosemia The enzyme has now been purified from liver [22] and can be used as a specific tool in the microdetermination of galactose 1 phosphate

*UDP galactose 4 epimerase* (III)

In a third reaction the two uridine nucleotides are directly interconverted




The enzyme catalyzing this reaction was previously called a Waldenase but with more recent data showing the requirement for diphosphopyridine nucleotide (DPN) [23] it is more appropriately called an epimerase Although definitive proof still needs to be obtained it appears that an epimerization occurs at carbon-4 of the hexose presumably by an oxidation reduction reaction The hypothetic 4 keto hexose intermediate has not been isolated but it seems highly probable that DPN functions here in the capacity of a hydrogen transporting agent

It is important to note that this key enzyme catalyzes a reversible reaction and that therefore UDP galactose can be formed from UDP glucose The latter is in turn derived from glucose 1 phosphate by the next reaction to be described (4) It is therefore evident that dietary galactose is not essential for UDP galactose synthesis and that the synthesis of such compounds as galactolipids may proceed from glucose via the epimerase despite the absence of Gal 1 P uridyl transferase

*UDP-glucose Pyrophosphorylase* (IV)

This enzyme is not directly involved in the galactose-glucose interconversion but is a means of synthesizing the important compound UDP glucose involved in the preceding two reactions The reaction is

which increases in activity with age [18] The development and presence of this pyrophosphorylase system in spite of the persistent deficiency of the transferase enzyme, would readily explain the improved galactose metabolism in the galactosemic person as he matures

## BIOCHEMICAL CONSIDERATIONS IN THE GALACTOSE-GLUCOSE INTERCONVERSION

Considerable knowledge has been gained in recent years concerning the metabolism of galactose and its utilization in the body Most of the ingested galactose is in the form of lactose the main carbohydrate of milk which in the intestine is split into its two component monosaccharides, galactose and glucose (Fig 7 2) These sugars differ only in the orientation of the hydrogen and hydroxyl groups about the fourth carbon atom The exact manner in which galactose is converted to glucose or glucose derivatives has been greatly elucidated in recent years principally as the result of investigations by Leloir Kalckar and their associates [19-21] The following enzymes and the reactions they catalyze are important and deserve consideration in the galactose-glucose interconversions

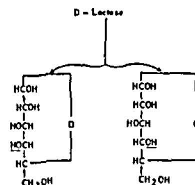



Fig 7 2 Comparison of the chemical structure of galactose and glucose

### *Galactokinase* (I)

This enzyme catalyzes the conversion of galactose to α-galactose-1-phosphate This requires adenosine triphosphate (ATP) in a reaction analogous to that catalyzed by hexokinase



Galactose + ATP ⟶ galactose 1 P + ADP (1)

The reaction is not reversible and adenosine diphosphate (ADP) is also formed The enzyme is known to exist in liver brain and red cells

### *Galactose 1 phosphate Uridyl Transferase* (II)

The conversion of galactose 1 phosphate to glucose 1 phosphate is now known to occur by a reaction which involves a specific nucleotide uridine diphosphoglucose (UDP glucose) In this reaction the galactose-1-phosphate is transferred to the nucleotide to yield uridine diphos-

## ENZYMATIC DEFECT IN GALACTOSEMIA

In studies aimed at elucidating the possible cause of galactosemia it seemed reasonable to assume that the defect might involve one or more of the above enzyme reactions Although the liver is the major site of the galactose glucose interconversion it was shown by Schwarz et al [25] that the red cells also metabolize galactose and that galactosemic red cells are abnormal in this respect These workers made the important observation that erythrocytes of galactosemic persons show an accumulation of galactose 1 phosphate after the ingestion of milk or galactose These observations have been confirmed with in vitro experiments in which galactosemic red cells incubated in a galactose-containing medium showed significant amounts of galactose 1 phosphate in contrast to normal red cells similarly incubated [17] Both these observations strongly suggested that the defect in galactosemia could be due to a block in the conversion of galactose 1 phosphate to glucose 1 phosphate and that such a block might reasonably involve the enzyme Gal 1 P uridyl transferase The occurrence of UDP galactose pyrophosphorylase in mammalian tissues was not known until recently but since it does not seem to exist in red cells knowledge of it would not have precluded focusing attention on the deficiency of Gal 1 P uridyl transferase as a plausible explanation for the in vivo and in vitro accumulations of galactose 1 phosphate

Biochemical studies of human erythrocytes aimed at elucidating the site of the abnormal galactose metabolism disclosed that these cells contain enzymes catalyzing Reactions 1 through 4 Galactosemic red cells were shown specifically to be deficient in enzyme II Gal 1 P uridyl transferase [17] (Table 7 2) This observation has been confirmed in all

TABLE 7 2 ENZYME ACTIVITY IN HEMOLYSATES OF NORMAL AND GALACTOSEMIC INDIVIDUALS

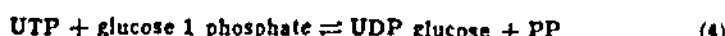
| Subjects | Galactokinase | | Gal 1 P uridyl transferase | | UDP galactose 4 epimerase | |
|---|---|---|---|---|---|---|
| | No | Activity | No | Activity | No | Activity |
| Normal | 3 | 0 10 | 15 | 0 8 | 3 | 0 3_ |
| Galactosemic | 3 | 0 09 | 10 | <0 0 | 3 | 0 35 |

Activity (average) is given in micromoles of reactants converted per milliliter lysed erythrocytes per hour

SOURCE K J Isselbacher et al Science 123 635 1956

the cases of galactosemia studied thus far It was shown that galactosemic red cells did not lack any cofactors nor did they contain any demonstrable inhibitors to account for the lack of transferase activity It was initially

reversible and requires uridine triphosphate (UTP) Pyrophosphate is formed as product of the reaction



UTP + glucose 1 phosphate ⇌ UDP glucose + PP (4)

This enzyme is abundant in liver red blood cells brain mammary gland and muscle

*UDP-galactose Pyrophosphorylase* (V)

Recent observations [27] indicate that mammalian liver also contains a pyrophosphorylase which catalyzes the following reaction



UTP + galactose 1 phosphate ⇌ UDP galactose + PP (5)

The activity of this enzyme in neonatal liver tissue is very weak but it has been found to increase with age Its activity (per milligram of protein)

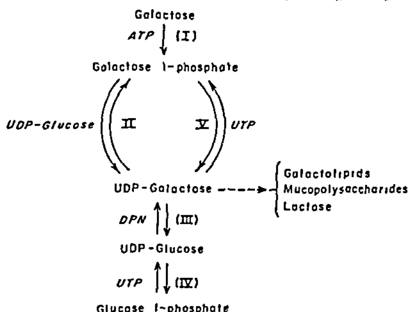



Fig 7 3 A schematic representation of the reactions and interactions important in the metabolism of galactose Roman numerals are the same as those in the text and refer to the specific enzymes catalyzing the various reactions Abbreviations include ATP adenosine triphosphate UDP-glucose uridine diphosphate glucose UDP galactose uridine diphosphate galactose UTP uridine triphosphate (*From K J Isselbacher Am J Med 26 715 1959*)

in adult liver is about one-sixth that of Gal 1 P uridyl transferase [18] It is apparent that this enzyme provides an additional pathway by which galactose 1 phosphate may be utilized and incorporated into UDP galactose The enzyme is found in liver and brain but has not been detected in erythrocytes

The interrelationships of these reactions are shown in Fig 7 3

From previous observations [17 25] one would expect that some of the retained galactose would be in the form of galactose 1 phosphate Studies performed with $C^{14}$ labeled galactose in a 24 year old male with galactosemia have permitted a more definitive analysis of this problem [27] The results are indicated in Table 7-4 It is observed that 3 per cent of the administered galactose could be accounted for in the urine as a glucosiduronic acid In view of present knowledge of the mechanism of glucosiduronic acid formation the data indicate that at least 3 per cent of the administered galactose must have been converted to glucose derivatives (via the uridine nucleotides) These observations suggest either that the deficiency of Gal 1 P uridyl transferase is incomplete in this disease or that there exists alternate or accessory pathway for galactose metabolism Since the enzymatic deficiency in the red cells is virtually complete the possibility of other routes of galactose utilization by passing the transferase reaction must be considered It is evident that

TABLE 7 4 METABOLISM OF C GALACTOSE BY AN ADULT PATIENT WITH GALACTOSEMIA

| *Determination* | *Amount mg* |
|---|---|
| Galactose in urine | 700 |
| Galactose metabolized to glucosiduronic acid | 30 |
| Galactose accumulating as cellular galactose (galactose 1 phosphate etc ) | 50–100† |

Values after infusion of 1 gm galactose and 5 microcuries galactose 1 C to a 4 year-old male with galactosemia 1 gm menthol was given orally

† All tissues with the exception of muscle and bone

SOURCE F Eisenberg Jr et al Science 125 116 1957

the enzyme UDP galactose pyrophosphorylase could function in such a manner and allow for galactose 1 phosphate utilization and incorporation into UDP galactose This theory is appealing because this enzyme has a very low activity in fetal and neonatal tissue but increases in activity with age [18] It is reasonable therefore to postulate that patients with galactosemia have their most pronounced symptoms at birth because at this time they have both an absent transferase system and low UDP galactose pyrophosphorylase activity The subsequent increase in activity of the latter enzyme with age would then explain the improved tolerance and galactose utilization which occurs as the galactosemic patient matures

## DIAGNOSTIC TESTS

### CHARACTERIZATION OF THE NATURE OF THE MELITURIA

When one finds a reducing sugar in an infant with or without the classic features of galactosemia described above it is imperative to establish

reasoned that the absence of the enzyme might simply be due to an adaptive phenomenon, i.e., since many of the galactosemic patients tested had been off galactose for extended periods of time, the red cells might gradually have lost their transferase activity. However, when erythrocytes of patients with milk allergy who had been off galactose for as long as 18 months were examined they were found to be normal with respect to all of the three other enzymes (I, III, V). Also it has been shown that the transferase deficiency exists at birth by demonstrating the absence of the transferase in the cord blood of infants afflicted with the disease [26].

The enzymatic defect noted in the erythrocytes has also been investigated in the liver tissue of two afflicted patients [26]. In order to estimate the activity of the enzyme in samples of liver tissue (biopsies) a different technique was necessary. Carbon 14 labeled galactose-1-phosphate was used and the rate of incorporation of radioactivity into the uridine nucleotide fraction was measured. This method is more sensitive than the spectrophotometric one used in the erythrocyte studies and allows detection of enzyme activity of as little as 1 per cent of the normal. It will be seen in Table 7-3 that the infant liver contained no detectable activity while the adult showed a rate of incorporation of $C^{14}$ galactose-1-phosphate which was 4 to 5 per cent of that in the nongalactosemic liver. It should be emphasized that this isotopic method is not so specific as the spectrophotometric one and would not distinguish between enzymes II and V. Therefore, the small activity in the adult galactosemic liver may be due to the enzyme UDP galactose pyrophosphorylase (V) (see below).

TABLE 7-3 URIDYL TRANSFERASE ACTIVITY IN HUMAN LIVER HOMOGENATES

| Subject | $C^{14}$ labeled Gal-1-P Incorporated* |
|---|---|
| Nongalactosemic adult (post mortem) | >15.0 |
| Nongalactosemic infant (post mortem) | >25.0 |
| Galactosemic adult (biopsy) | 1.2 |
| Galactosemic infant (biopsy) | <0.3 |

* Activity in micromoles of label incorporated per gram of liver per hour.
SOURCE: E. P. Anderson et al., Science, 125, 113, 1957.

## POSSIBLE ALTERNATE PATHWAYS OF GALACTOSE METABOLISM IN GALACTOSEMIA

As indicated above, observations have been made by several groups of investigators which suggest that administered galactose can be metabolized to some degree in galactosemic patients [3]. These conclusions have been based on the fact that from 30 to 80 per cent of ingested galactose is retained and not accounted for by urinary excretion of the sugar

GALACTOSE 1 PHOSPHATE ACCUMULATION IN ERYTHROCYTES

Schwarz Holzel and Komrower have advocated the use of an in vitro test based on the accumulation of galactose 1 phosphate in galactosemic red cells [32] In this test the red cells are incubated with galactose Phosphorylated derivatives are then isolated as their barium salts The sugar phosphates are hydrolyzed and the free sugars separated by paper chromatography The galactose is estimated by comparison with known quantities chromatographed simultaneously The main disadvantage of this test is that it requires 2 to 3 days to carry out As with the enzymatic procedure this test can be performed on cord blood and the diagnosis made several days after birth

## POSSIBLE FACTORS IN TOXICITY

Although galactosemia has been observed only in man somewhat analogous physiologic changes can be induced in animals by feeding diets high in galactose It has been observed repeatedly that when chicks are placed on 30 per cent galactose diets they may show a quivering syndrome [33] and rats uniformly develop cataracts within a period of 14 to 21 days [34] In the galactose-fed rats with cataracts examination of the lenticular tissues [34] has revealed significant amounts of galactose 1 phosphate just as in the galactosemic red cells It is tempting to speculate that this hexose-phosphate accumulation may interfere with or inhibit one or more enzyme systems

Experimental evidence to date bears out some of these speculations Thus it has been recently reported [35 36] that galactose 1 phosphate *inhibits* phosphoglucomutase provided that glucose 1 6-diphosphate is not present in excess The physiologic significance of this interference is at present difficult to evaluate but it may be another factor contributing to the hypoglycemia which occurs in galactosemic patients when they ingest galactose or lactose

Recently Lerman [36a] has indicated that there seems to be an inhibition of glucose 6-phosphate dehydrogenase in the lens of rats fed on high galactose diets It was suggested that galactose 1 phosphate accumulation in the cataract was responsible for this inhibition The author [36b] has studied the activity of purified glucose 6 phosphate dehydrogenase of red cells* and has found no inhibition at concentrations up to ten times that of the substrate glucose 6-phosphate It would seem therefore that the observations of Lerman do not seem valid for the glucose 6-phosphate dehydrogenase of tissues other than the lens

The consumption of ATP in the phosphorylation of galactose to galactose 1 phosphate in these patients could conceivably also result in

* Kindly supplied by Dr Paul A Marks

the identity of the sugar Recently this has been greatly facilitated by methods utilizing the enzyme glucose oxidase Commercial test preparations containing glucose oxidase such as Tes tape and Clinistix will give a positive reaction only in the presence of glucose [1] thus nonglucose sugars can readily be suspected A recently reported method [29] using a microbial galactose oxidase is promising since it will permit rapid and definitive demonstration of galactose in biologic fluids In addition to the usual chemical methods for identifying galactose (e g oxidation of the sugar to mucic acid) paper chromatography has been employed to assist in the diagnosis [30]

### GALACTOSE TOLERANCE TEST

In the past it has been customary to confirm the diagnosis of galactosemia by means of the oral or intravenous galactose tolerance test [1 3] This test is not without hazard especially in an infant in whom there is marked impairment of galactose utilization These patients often develop hypoglycemia during the course of the test (Fig 7 1) and serious reactions such as convulsions may occur For this reason and because infants with galactosemia should preferably not be exposed to galactose *the galactose tolerance test should be avoided if at all possible* especially since one can now make the diagnosis by other tests cited below which do not jeopardize the health of the infant

### ERYTHROCYTE ASSAY FOR GAL 1 P URIDYL TRANSFERASE

As a result of the observations that circulating erythrocytes normally contain abundant amounts of Gal 1 P uridyl transferase a test has been developed which is based on the absence of this enzyme in galactosemia This method the details of which have been outlined elsewhere [31] is both sensitive and specific No false positives have been encountered Patients with diseases such as hepatitis or cirrhosis in whom galactose tolerance is abnormal do not demonstrate a defect of transferase activity in their red cells Errors may occur if a galactosemic patient has had a transfusion of normal blood within a period 3 months prior to the assay In such a situation the transferase content of the transfused normal cells may give a false result [26] This enzymatic method has a number of advantages (1) it is quickly performed and the result can be obtained in several hours (2) it permits diagnosis at birth on umbilical cord blood a feature desirable if the newborn infant is a member of a galactosemic family (3) it avoids the hazards of galactose administration (4) it is specific

[1] Some purified fungal preparations of glucose oxidase have been reported to oxidize galactose to a small extent [28] This does not seem to be a problem with the enzyme used in Tes tape and Clinistix

A method much more suitable for genetic studies has recently been developed by Kirkman and Bynum [41] Their assay is a manometric one using hemolyzates of human erythrocytes and is carried out in such a manner that zero-order kinetics obtain In a study of 18 parents of galactosemic patients all parents possessed enzymatic activity below the normal mean Thirteen of the 18 parents had activities which were more than two standard deviations below the normal mean Some of their data are reproduced in Fig 7-4 It is apparent that parents as well as normal

GALACTOSEMICS
PARENT
NORMALS
0 8 16 24 32 40
G 1 P URIDYL TRANSFERASE ACTIVITY

Fig 7-4 A comparison of the galactose 1 phosphate uridyl transferase activities in galactosemic patients their parents and normal controls Activity is expressed as microliters of oxygen uptake per hour for 0 3 ml Represents a galactosemic patient after transfusion with normal blood (*By permission of H N Kirkman et al* [41])

persons vary somewhat in the amount of Gal 1 P uridyl transferase activity and parents and the normal controls are seen to overlap slightly in their upper and lower extremes It is possible as these authors point out that the variation in the observed enzymatic activity represents a combination of environmental factors laboratory error and possibly even other genes but that nevertheless all parents are heterozygous and possess diminished transferase activity

Recently Weinberg Herring Johnson and Field [43] have employed the oxidation of galactose 1 $C^{14}$ to $C^{14}O_2$ by leukocyte suspensions or whole blood to study patients with galactosemia They have observed that there is only negligible production of $C^{14}O_2$ by cells from galactosemic individuals when incubated with $C^{14}$ labeled galactose With this sensitive technique they were able to detect and quantitate the carrier state in one family

Using this method the author has investigated the problem of whether the enzymatic defect in galactosemia is complete or only partial The spectrophotometric assay for galactose 1 phosphate uridyl transferase has been very suggestive of a complete defect but the sensitivity of the assay is such that demonstration of the complete absence of the enzyme has not been possible

The experiments performed were based on the observation that the oxidation of galactose by erythrocytes is markedly enhanced (ten to

cellular disturbances Thus the cells would be yielding a significant amount of their ATP to form a product (galacto e 1 phosphate) which accumulates and is not readily metabolized Of relevance in this connection are observations by Pennington and Prinkerd [37] that the ATP content of a galactosemic patient s red cells decreased when the individual was placed on a lacto e diet

The hypoglycemia which may occur in this di ease has also been considered by many as causally related to the clinical manifestations of the disease, especially in regard to the mental retardation It should be recalled that the symptoms and signs found in galactosemia notably the lenticular and central nervous system changes do not occur in uch conditions as von Gierke s disease where episodes of hypoglycemia are pronounced and evere

## GENETIC CONSIDERATIONS

A number of the inborn diseases of metabolism have been shown to result from a combination of two recessive genes Phenylpyruvate oligophrenia is a typical example of a disease transmitted as a single recessive trait parents of the afflicted patients show a distinctly lowered tolerance for phenylalanine [38]

The frequent occurrence of galactosemia in siblings and among the offspring of consanguineous matings [11] together with its equal incidence in both sexes suggests that this disea e is transmitted by a single autosomal reces ive gene Holzel and Komrower [39] have investigated the genetics of galactosemia u ing the oral galactose tolerance test They found mild impairment of galactose tolerance in some relatives but were unable to show abnormal tolerance in *both* parents It is difficult to reconcile this observation with the concept that galactosemia is a simple recessive disease in which *both* parents should be heterozygous It is apparent that this test is not sufficiently sensitive to allow definitive conclusions concerning the genetic mode of inheritance in galacto emia This same limitation applies to the use of the Gal 1-P uridyl transferase assay for genetic studies Although this test is reliable and specific the kinetics of the method are such as to make it difficult to assay quantitatively intermediate amounts of the enzyme Nevertheless Hsia et al [40] have used the transferase assay in genetic studies In a preliminary report of their observations on five galactosemic families they described decreased Gal 1 P uridyl transferase activity in heterozygotes It should be noted that the differences in values between their normal controls (4 5 ± 0 47 units per gm hemoglobin) and the heterozygotes (3 3 ± 0 36) make it difficult to interpret the results in any single individual Kalckar et al were unable to find any significant differences between parents and normal controls using the transferase assay [31]

2 The disease is transmitted by a single autosomal recessive gene and is expressed as a characteristic cellular deficiency of the enzyme galactose 1 phosphate uridyl transferase

3 The clinical manifestations consist of nutritional failure hepatosplenomegaly with cirrhosis cataracts and mental retardation The major laboratory findings are galactosuria aminoaciduria albuminuria and an impaired galactose tolerance test result All these manifestations may disappear or regress if galactose is removed from the diet

4 The cause and mechanism of the toxic manifestations are not clear They appear to be related to the accumulation in the tissues of galactose 1 phosphate

5 The presence of galactose 1 phosphate uridyl transferase in normal erythrocytes and its absence in galactosemic cells have provided the basis for rapid and specific diagnostic tests for this condition

6 Some improvement in galactose utilization occurs in these patients as they mature This may be related to the activity of an accessory pathway of galactose metabolism via the enzyme uridine diphosphogalactose pyrophosphorylase

## BIBLIOGRAPHY


1 Townsend E H Mason H H and Strong L S Galactosemia and its relation to Laennec s cirrhosis review of literature and presentation of six additional cases Pediatrics 7 760 1951
2 Ritter J A and Cannon E J Galactosemia with cataracts report of a case with notes on physiopathology New England J Med 252 747 1955
3 Komrower G M Schwarz V Holzel A and Goldberg L A clinical and biochemical study of galactosemia Arch Dis Childhood 31 254 1956
4 Foa P P Galansino G Costa E and Pozza G Regulation of insulin secretion by carbohydrates other than glucose Fed Proc 15 64 1956
5 Bergren W R Connell G and Kalckar H M Congenital galactosemia and mental health Lancet 1 267 1958
6 Holzel A Komrower G M and Wolson V K Aminoaciduria in galactosemia Brit M J 1 194 195
7 Hsia D Y Y Hsia H Green S Kay M and Gellis S S Amino aciduria in galactosemia A M A Am J Dis Child 88 458 1954
8 Darling S and Mortensen O Amino aciduria in galactosemia Acta paediat 43 337 1954
9 Komrower G M L aminoacidurie dans la galactosemie Arch franç pédiat 10 185 1953
10 Cusworth D C Dent C E and Flynn F V The aminoaciduria in galactosemia Arch Dis Childhood 30 150 1955
11 Holzel A Komrower G M and Schwarz V Galactosemia Am J Med 22 703 1957
12 Holzel A Komrower G M and Schwarz V Low lactose milks for galactosemia Lancet 2 92 1955
13 Platt B S Human nutrition and the sophistication of food and feeding habits Brit M J 1 179 1955
14 Kalckar H M Biochemical mutations in man and microorganisms Science 125 105 1957
15 Burton R M Sodd M A and Brady R O The incorporation of galactose into galactolipids J Biol Chem 233 1053 1958

twentyfold) by an electron acceptor dye such as methylene blue at a concentration of $10^{-4}M$. The effect is comparable to that observed by Brin and Yonemoto [44] with glucose. In the presence of a partial deficiency of uridyl transferase one would expect some stimulation of galactose oxidation although perhaps diminished. However in the presence of a complete enzyme lack no enhanced oxidation should occur. It has been observed (Table 7.5) that the red cells from parents of galactosemic patients have a reduced stimulation with methylene blue (sixfold increase) while cells from the homozygous individuals show no response to the addition of dye. These findings strongly suggest that at least in the red cell the enzymatic defect is complete.

TABLE 7.5. THE OXIDATION OF GALACTOSE-1-$C^{14}$ TO $C^{14}O_2$ AND THE EFFECT OF METHYLENE BLUE BY RED CELLS FROM NORMAL SUBJECTS AND IN HOMOZYGOTES AND HETEROZYGOTES FOR GALACTOSEMIA

| *Subject* | *$C^{14}O_2$ ... at 1 per 100 mg Hb* | *Per cent stimulation of $C^{14}O_2$ oxidation by methylene blue $10^{-4}M$* |
|---|---|---|
| Normal (5) | 11 800 | 1 [illegible] |
| R. D. Mother | 5 [illegible] | 538 |
| F. D. Father | 8 740 | 57[illegible] |
| I. D. 1 yr galactosemic | 290 | 1 |
| C. D. 4 yr galactosemic | [illegible]0 | 3 |

The incubation system contained 0.5 ml washed red cells, 0.25 microcurie galactose-1-$C^{14}$, methylene blue $10^{-4}M$ and [illegible] buffer [44] to a total volume of 1.[illegible] ml. Incubation was for 90 min at 37° C.

Most recently Bretthauer et al. [42] have successfully employed a modified method of assay of Gal-1-P uridyl transferase in which hemolyzed red cells are briefly incubated with optimal amounts of Gal-1-P and UDP Glu. Segregation of galactosemic patients, presumed heterozygotes and normal subjects appeared to be complete.

It still is not known whether these heterozygous individuals have a reduced amount of unaltered and structurally *normal* enzyme or whether the lowered activity which they demonstrate is the result of some *hybrid* gene product. These and other considerations as they apply to biochemical genetics have been admirably reviewed by Kalckar [45].

## SUMMARY

1. Galactosemia is a congenital and hereditary disease of carbohydrate metabolism characterized by an impairment in the ability to metabolize and utilize ingested galactose or galactose-containing compounds.

40 Hsia D Y Y Huang J and Driscoll S G Heterozygous carrier in galactosemia Nature London 182 1380 1958
41 Kirkman H N and Bynum E Enzymatic evidence of a galactosemic trait in parents and galactosemic children Ann Human Genet 23 117 1959
4 Bretthauer R K Hansen R C Donnell G and Bergren W R A procedure for detecting carriers of galactosemia Proc Nat Acad Sc (U S) 45 328 1959
43 Weinberg A N Herring B Johnson P and Field J B Galactose metabolism by leukocytes from galactosemic patients their parents and normals Clin Res 8 27 1960
44 Brin M and Yonemoto R H Stimulation of the glucose oxidative pathway in human erythrocytes by methylene blue J Biol Chem 230 307 1958
45 Kalckar H M Some considerations regarding biochemical genetics in man Perspectives in Biol & Med 1 3 1957

16 Kalckar H M Anderson L I and Isselbacher K J Galactosemia a congenital defect in a nucleotide transferase Biochim et biophys acta 20 262 1956
17 Isselbacher K J Anderson E P Kurahashi K and Kalckar H M Congenital galactosemia a single enzymatic block in galactose metabolism Science 123 635 1956
18 Isselbacher K J Evidence for an accessory pathway of galactose metabolism in mammalian liver Science 126 652 1957
19 Leloir L F The metabolism of hexose phosphates in *Phosphorus Metabolism* edited by W D McElroy and B Glass vol I p 67 Johns Hopkins Press Baltimore 1951
20 Leloir L F Enzymatic transformation of uridine diphosphate glucose into galactose derivative Arch Biocem 33 186 1951
21 Kalckar H M Braganca B and Munch Petersen A Uridyl transferases and the formation of uridine diphosphate galactose Nature London 172 1038 1953
22 Kurahashi K and Anderson E P *Galactose 1 phosphate uridyl transferase* its purification and application Biochim et biophys acta 29 498 1958
23 Maxwell E S The enzymic conversion of uridine diphosphogalactose and uridine diphosphoglucose J Biol Chem 229 139 1957
24 Isselbacher K J A mammalian uridine diphosphate galactose pyrophosphorylase J Biol Chem 232 429 1958
25 Schwarz V Goldberg L Komrower G M and Holzel A Some disturbances of erythrocyte metabolism in galactosemia Biochem J 62 34 1956
26 Anderson L I Kalckar H M and Isselbacher K J Defect in the uptake of galactose-1 phosphate into liver nucleotides in congenital galactosemia Science 125 113 1957
27 Eisenberg F Jr Isselbacher K J and Kalckar H M Studies in the metabolism of carbon [14]-labeled galactose in a galactosemic individual Science 125 116 1957
28 Waldstein S S Arcilla R A and Dubin A The effect of purified glucose oxidase on galactose Quart Bull Northwestern Univ M School 31 373 1957
29 Cooper J A D Galactose oxidase from *Polyporus circinatus* Fr J Biol Chem 234 445 1959
30 Bickel H and Hickmans E M Paper chromatographic investigations on the urine of patients with galactosemia Arch Dis Childhood 27 348 1952
31 Anderson E P Kalckar H M Kurahashi K and Isselbacher K J A specific enzymatic assay for the diagnosis of congenital galactosemia J Lab Clin Med 50 569 1957
32 Schwarz V Holzel A and Komrower G M Laboratory diagnosis of congenital galactosemia at birth Lancet 1 24 1958
33 Hansen R G Freedland R A and Scott H M Lactose metabolism V The uridine nucleotides in galactose toxocity J Biol Chem 219 391 1956
34 Schwarz V and Golberg L Galactose-1 phosphate in galactose cataract Biochim et biophys acta 18 310 1955
35 Sidbury J B Jr The enzymatic lesions in galactosemia J Clin Invest 36 929 1957
36 Ginsburg V and Neufeld E F Inhibition of phosphoglucomutase by galactose-1 phosphate a possible factor in galactose toxicity p 7c Abstract of papers presented at the National Meeting of the American Chemical Society New York 1957
36a Lerman S Enzymatic factors in experimental galactose cataract Science 130 1473 1959
36b Isselbacher K J Unpublished observations
37 Pennington J S and Prankerd T A J Studies of erythrocyte phosphate ester metabolism in galactosemia Clin Sc 17 385 1958
38 Hsia D Y Y Driscoll K W Troll W and Knox W E Detection by phenylalanine tolerance tests of heterozygous carriers of phenylketonuria Nature London 178 1239 1956
39 Holzel A and Komrower G M A study of the genetics of galactosemia *Arch Dis Childhood* 30 155 1955

bilirubin in biologic specimens [12] When icteric serums were studied with this method it soon was recognized that bilirubin appeared to be present in two different forms one gave a *direct* reaction when diazotized sulfanilic acid was added to icteric serum whereas the other coupled with the diazo reagent only after alcohol was also added to the reaction mixture (*indirect* reaction) Separate determination of these two pigment fractions was found to be of clinical usefulness in the differential diagnosis of jaundice [13] For many years the basic reason for this difference in reaction type was unknown and numerous explanations and hypotheses were offered [14]

The discovery that bilirubin is excreted in conjugated form [15–17] provided a simple explanation for this problem Conjugated bilirubin is water soluble and reacts with diazotized sulfanilic acid in the *absence* of alcohol (*direct* van den Bergh reaction) whereas native bilirubin is *insoluble* in water [18] and requires addition of solvents to permit coupling with the diazo reagent (*indirect* van den Bergh reaction)

The finding that for excretion in the bile bilirubin is converted to water soluble conjugates throws new light on the way by which the pigment is handled in the liver The individual steps of this process may be visualized as follows

1 Transport of bilirubin from the plasma into the liver cells

2 Localization of bilirubin at the surface of or penetration into liver cell microsomes

3 Conjugation of bilirubin by the microsomal enzyme system

4 Transport of conjugated bilirubin from the hepatic cells into the bile

A defect in one or more of the first three steps would result in decreased formation of conjugated bilirubin and hence in retention of *unconjugated* bilirubin in the plasma A disturbance of the fourth step would lead to impaired excretion of *conjugated* bilirubin resulting in its regurgitation into the plasma

In the following discussion a number of syndromes will be considered in which icterus appears to be due to one or a combination of several such defects It should be stated at the onset however that in most instances direct and unequivocal demonstration of the precise nature of the defect(s) has not yet been possible For the most part the argument is indirect or by analogy Moreover clear evidence for genetic control of these metabolic abnormalities is available only in some of the syndromes while in others inheritance may be suspected but has not yet been proved

## FORMATION AND METABOLISM OF BILIRUBIN

### BREAKDOWN OF HEMOGLOBIN

The average life span of the normal human erythrocyte is 120 days [19] At the end of this period the cell is removed from the circulation and its

# Chapter 8

# Hyperbilirubinemia*

*Rudi Schmid*

Jaundice as a manifestation of liver disease has been known for centuries. Its diagnostic and prognostic aspects were discussed by Hippocrates [1] and its association with the presence of gallstones in the biliary tract was recognized by Galen [2].

The concept that *bile* pigment is derived from *blood* pigment is probably very old, but the first clear and experimentally supported indication was provided by Virchow [3], who observed and isolated bilirubin crystals from old blood extravasations. Through many classical experiments conducted over the following years unmistakable evidence accumulated that bilirubin is derived from the prosthetic group of hemoglobin [4-9]. The final proof was obtained much later when Fischer and his school elucidated the *chemical* constitution of heme and of bilirubin and demonstrated that both have a closely related tetrapyrrolic structure [10].

For a long time the anatomic site of bilirubin formation in the organism was a matter of much controversy. On the basis of the findings of Minkowski and Naunyn [11] that in hepatectomized geese induced hemolysis failed to produce jaundice, it was thought that the liver was the only organ capable of converting hemoglobin to bilirubin. This was an unfortunate interpretation of a correct observation, later clarified by the finding that in the goose virtually all reticuloendothelial cells are located in the liver. In mammals McNee [6], Mann [8], Rich [7] and others obtained conclusive evidence that formation of bilirubin *does* occur following total hepatectomy. As a result of these findings the view gained general acceptance that hemoglobin is converted to bilirubin in the entire reticuloendothelial system; the pigment is then transported in the blood to the liver, where it is excreted in the bile.

Quantitative studies of bile pigment metabolism were greatly facilitated when van den Bergh introduced Ehrlich's diazo reaction for estimation of

* This work was supported in part by grant A 1833(C) from the Public Health Service.

Conversion of hematin to bile pigments has been demonstrated in vivo and in vitro [8 30–34] The earlier belief that this occurs by formation of hematoporphyrin or protoporphyrin as intermediates has been shown to be erroneous [31] Kench et al [33] failed to observe bile pigment formation in vitro by coupled oxidation of protoporphyrin and L-ascorbic acid whereas under the same conditions positive results were obtained with hematin methemalbumin hemoglobin and methemoglobin These findings and those reported by Fischer [35] Libowitzky [34] and Lemberg [36] suggest that the presence of a divalent or trivalent iron may be

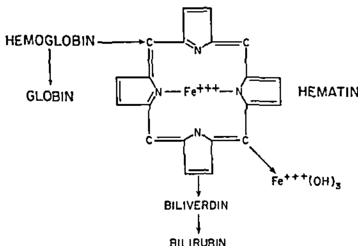



FIG 8-2 Hemoglobin catabolism The exact nature of the breakdown of hemoglobin is not fully understood According to one view the first intermediate formed is hematin

essential for the cleavage of the protoporphyrin ring Recent studies in vivo [37] which originally were believed to demonstrate low grade conversion of isotopically labeled protoporphyrin to stercobilin do not contradict this concept since it is likely that a small fraction of the administered protoporphyrin is first converted to heme before being broken down to bile pigments [24] It is well established that protoporphyrin may undergo spontaneous coordination with ferrous iron to form heme [38 39] With the exception of a photochemical reaction requiring very special conditions [40] there is no evidence to suggest that free protoporphyrin can be directly converted to bile pigments in vivo or in vitro

Lemberg and his school have challenged the concept that hematin is an intermediate in the physiologic breakdown of hemoglobin to bile pigments [36] Coupled oxidation of hemoglobin and ascorbic acid in vitro was found to result in formation of choleglobin a green bile pigment iron globin complex which upon treatment with acetic acid yields

hemoglobin is degraded [10] The factors responsible for the "aging of circulating red blood cells are poorly understood Destruction of old erythrocytes takes place in the reticuloendothelial cells [19] but it is not known to what extent the different organs containing the reticuloendothelial cells participate in this process Under conditions of abnormally increased erythrocyte destruction cells may be destroyed predominantly either in the spleen or in the liver, depending upon the type of damage to which the erythrocytes are subjected [20] The problems relative to the site of red cell destruction and bilirubin formation have been reviewed by Lepehne [21] With [14] and Watson [13]

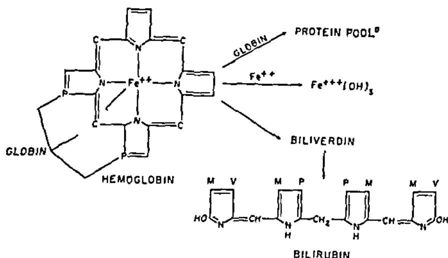



Fig 8-1 Hemoglobin catabolism Of the three hemoglobin constituents the iron and the globin amino acids are reutilized whereas the protoporphyrin is converted to bile pigment and excreted

Of the three hemoglobin constituents iron is almost completely reutilized for formation of new iron containing compounds [22] The globin is probably degraded and returned to the amino acid pool (Fig 8-1) [19] The protoporphyrin moiety is not preserved the porphyrin ring is opened and the resulting bile pigment is excreted (Fig 8-1) [23 24] The exact nature and the sequence of the individual steps in the catabolism of hemoglobin are still matters of controversy

It has been assumed that the first intermediate formed is hematin According to this view the initial step consists in splitting of the iron protoporphyrin complex from the globin (Fig 8 2) [2 13 25 26] In conditions associated with intravascular hemolysis [27] and in some instances of severe liver disease [13 28] the plasma may contain hematin bound to albumin (methemalbumin) [29] Under physiologic conditions hematin cannot be demonstrated in the blood presumably because the breakdown of hemoglobin occurs inside the reticuloendothelial cells [2,]

there are other metabolic products of hemoglobin [24] A significant discrepancy has occasionally been noted between the estimated rate of hemoglobin breakdown and the amount of bile pigments recovered [14, 50–52] This is illustrated by patients suffering from severe congenital nonhemolytic jaundice of the type described by Crigler and Najjar [53] (see below) Children with this disorder have a reduced capacity to form conjugated bilirubin and hence to excrete the pigment causing marked retention of bilirubin in the plasma [54, 55] The bile although otherwise normal may be almost totally devoid of bile pigments and fecal urobilinogen excretion is very low [54] In spite of nearly complete inability to excrete bilirubin the degree of jaundice is not steadily increasing but remains essentially stationary [24] This would not be expected if bilirubin were the only metabolic end product of hemoglobin breakdown It is possible that heme or bilirubin or both may be degraded to and excreted as di or monopyrrole but detection and isolation of these compounds is technically difficult

Dipyrrolic compounds belonging to the group of bilifuscin meso bilifuscin and pentdyopent have been identified in and isolated from the feces of healthy individuals [56–58] Pentdyopent has occasionally been found in the urine of jaundiced patients [59, 60] However the metabolic origin of these substances has not yet been established with certainty [58]

In addition dark pigments belonging to the mesobilifuscin group are excreted in the urine of patients with a rare congenital syndrome in which increased hemolysis is associated with the presence of erythrocyte inclusion bodies [61] In these instances the urinary pigments are believed to be derived from the abnormal red cell but the nature of the metabolic defect in the erythrocytes is not yet known

With [62] and Lups and Meyer [63] suggested that dipyrroles of the bilifuscin type occur in normal and in jaundiced serums but direct evidence to support this claim was not obtained Exposure of hemoglobin to hydrogen peroxide in vitro [64] or coupled oxidation with unsaturated fatty acids [65] results in formation of dipyrrolic fragments There is no convincing evidence that such mechanisms are of physiologic significance [2]

These scattered observations indicate that the exact mechanism of hemoglobin breakdown is still poorly understood but there can be little doubt that bilirubin and the pigments and chromogens that result from its further alteration in the intestine represent the major end products of heme catabolism [2, 24, 36]

### FORMATION OF BILE PIGMENTS FROM SOURCES OTHER THAN MATURE ERYTHROCYTES

Attempts to correlate bilirubin excretion in the bile with the rate of erythrocyte destruction as determined by other methods suggested that 80 to 100 per cent of the degraded hemoglobin can be accounted for as

biliverdin and free iron (Fig 8-3) [36] According to their view the porphyrin ring is opened while the heme group is still attached to the protein moiety Recently Mills [41] and Kaziro et al [42] confirmed and extended these observations and suggested that in circulating erythrocytes protective mechanisms may prevent excessive formation of choleglobin during life Small amounts of choleglobin like pigments have been observed in normal rabbit erythrocytes [43] and in stored human red cells [44] as well as in red cells of rabbits treated with phenylhydrazine [45] It has been suggested that the so-called easily split iron of erythrocytes may actually represent that portion of the red cell iron which is part of the choleglobin [46]

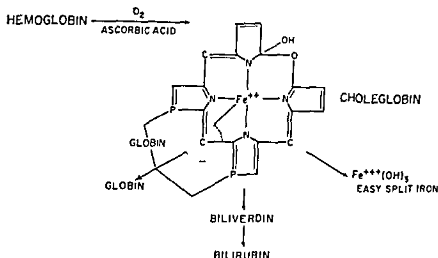



Fig 8-3 Hemoglobin catabolism Another proposed mechanism for the breakdown of hemoglobin assumes that the first intermediate formed is choleglobin which is a green bile pigment iron globin complex in which the porphyrin ring has been opened in one of the methene bridges

The demonstration of choleglobin like compounds in red cells is technically difficult because of the large excess of hemoglobin present Whether these green pigments may simply represent an artifact is still a matter of considerable controversy [33 47-49] This question cannot be resolved at present The finding of Kench et al [33] that hematin can be converted in vitro to bile pigments makes it unnecessary to postulate that the opening of the prophyrin ring must take place while heme is still attached to protein On the other hand the fact that hematin may be converted to bile pigments is not proof that the former is a true intermediate in the breakdown of hemoglobin to bilirubin

Whatever the physiologic pathway of hemoglobin catabolism may be either with hematin or with choleglobin as intermediate it appears certain that bilirubin is the principal bile pigment formed [2 36] There is at least suggestive evidence however that in addition to bilirubin

globin of mature circulating erythrocytes but must originate from other sources It has been suggested that bile pigment may be formed from hemoglobin of developing erythrocytes which are destroyed in the marrow or shortly after they reach the circulation [67 68] it is also possible that immature red cells may be *forming* as well as *degrading* hemoglobin [68] or that heme formed in excess of globin may be degraded to bile pigment [68] Direct synthesis and excretion of bile pigments appears less likely but cannot be excluded with certainty

A small fraction of excreted stercobilin is undoubtedly derived from heme compounds other than hemoglobin such as myoglobin catalase peroxidase and cytochromes The quantity of these chromoproteins in the organism is relatively small and little is known of their rate of turn over [69] It has been suggested that nonhemoglobin heme may be the source of the small but significant amount of labeled stercobilin which is excreted between the thirtieth and eightieth day [67 68] following the administration of glycine-$N^{15}$ (Fig 8-4) Since during this period there is no apparent decrea e in the isotope concentration of circulating red cell hemoglobin it appears unlikely that hemoglobin is the source of this bile pigment fraction

Quantitative analysis of the pattern of labeled stercobilin excretion in normal individuals suggests that approximately 70 per cent of the excreted bile pigment originates from destruction of mature red cells which have reached the end of their physiologic life span [67 68] An additional 10 to 20 per cent of pigment is excreted during the first week and comprises the initial peak [67 68] This fraction although probably not derived from hemoglobin of mature circulating erythrocytes appears in some way to be related to formation or maturation of erythroid cells in the bone marrow [70] The excretion of labeled stercobilin during the period between the thirtieth and the eightieth day amounts to less than 10 per cent of all bile pigments formed and probably reflects to a large extent turnover of heme compounds of nonhemoglobin nature

In diseases associated with abnormalities in erythropoiesis the pattern of bile pigment excretion may differ significantly from that found in healthy individuals Thus in congenital erythropoietic porphyria at least 31 per cent [71] and perhaps as much as 80 per cent [68] of the excreted stercobilin is not derived from hemoglobin of mature circulating erythrocytes Likewise in pernicious anemia 40 per cent or more of the fecal bile pigment seems to be derived from sources other than mature red blood cells [72]

These findings indicate that the excreted bile pigments are derived *from multiple* metabolic sources the most important of which is undoubtedly hemoglobin in circulating erythrocytes On the other hand estimation of pigment excretion in bile or feces may fall short of the *total* turnover of heme compounds in the body ince alternative pathways

bile pigments [66] This concept, based on studies carried out *before* the advent of tracer techniques requires modification now that it is known that a significant fraction of the excreted bile pigment is derived from sources other than hemoglobin of aged erythrocytes

When glycine-$N^{15}$ is administered to a normal person and the concentration of the labeled nitrogen is determined in the excreted bile

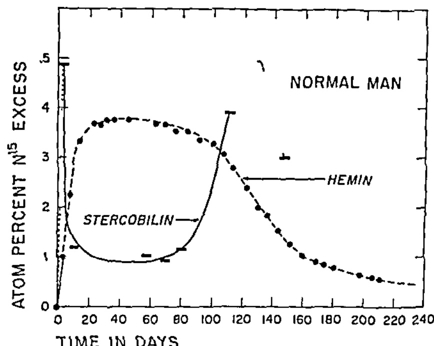
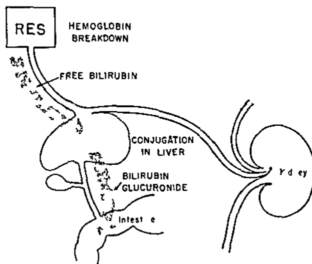



FIG 8-4 Formation of bile pigment in normal man When glycine $N^{15}$ is administered to normal man hemin of circulating hemoglobin shows a rapid increase in isotope content Fecal stercobilin exhibits two separate peaks of high isotope concentration a first maximum is reached during the first week while the second maximum coincides with the period of maximal breakdown of labeled erythrocytes (*By permission of I M London et al [67]*)

pigments over the following 150 days a curve with two separate peaks is obtained, an initial smaller peak is reached during the first week after the isotope administration and a second larger peak occurs after approximately 130 days [67 68] (Fig 8-4) The *second* peak coincides with the period of maximal breakdown of labeled erythrocytes (Fig 8-4) suggesting that *this* fraction of fecal stercobilin is derived from mature red cells which are destroyed at the end of their physiologic life span

The initial peak in $N^{15}$ labeled stercobilin occurs at a time when the $N^{15}$ of hemoglobin in circulating red cells is still increasing (Fig 8-4) This fraction of stercobilin could not therefore be derived from hemo-

curonic acid is attached to the carboxyl groups of the pigment to form an acyl glucuronide (Fig 8 6) which is unstable at alkaline pH [83 84] Because of its water solubility conjugated bilirubin exhibits a *direct* van den Bergh reaction while unconjugated bilirubin couples with the diazo reagent only after addition of alcohol or of another suitable solvent (*indirect* van den Bergh reaction) [81 82] It should be noted, however that in plasma a small fraction of the unconjugated pigment may react with the diazo reagent in the *absence* of alcohol [14 82] This is probably because of the presence of solubilizing substances such as bile acids urea




Fi 8-6 In man and otl r mammals b lirul in is excrete l in conjugated form mainly as a glu uronide

and citrate [14] For this reason the direct and indirect v in den Bergh reactions do not permit an exact estimation but only an *approximation* of the concentration of conjugated and unconjugated bilirubin present [82]

Many metabolites and several exogenous compounds admini tered for di gno tic or ther ipeutic reasons are excreted as glucuronide Glucuronide formation provides the org ini m with a mechani m by which harmful or metabolically active ub t inces can be detoxified or inactivated Synthe is of glucuronides is catalyzed by an enzyme system pre ent in liver microsomes [85–87] It is not yet known whether a ingle enzyme or several related micro omal enzyme are involved in the bio ynthesi of different types of glucuronides [88] In all instances the source of the glucuronic acid moiety is the nucleotide uridine dipho phate glucuronic acid (UDPGA) formed by a dehydr genase reaction catalyzed by a

of catabolism may exist and may yield metabolic products not detected with the available methods [24]

### TRANSPORT, CONJUGATION, AND EXCRETION OF BILIRUBIN

The first bile pigment formed in the reticuloendothelial cells is probably biliverdin [2 36] which is rapidly reduced to bilirubin The bile of many species of Amphibia and birds contains only biliverdin [2] but mammals excrete predominantly bilirubin [36] Reduction of biliverdin to bilirubin in vitro has been demonstrated in a variety of mammalian tissues including liver, kidney and spleen [36, 37]

The exact mechanism by which bilirubin is released from the reticuloendothelial system into the plasma is not clear Since the pigment is *insoluble* at the pH of the blood [18] it must be carried by attachment to proteins The vast and contradictory literature relating to the mode of transport of bilirubin in the plasma has been reviewed by With [14] It is generally agreed that in jaundiced serums most of the bilirubin is bound to albumin [14] but attachment of bilirubin to other plasma proteins has also been demonstrated [74] Cohn [75] has isolated a pure $\alpha_1$ globulin belonging to the V 1 fraction which strongly binds bilirubin This fraction was estimated to constitute less than 0 1 per cent of all plasma proteins [7o]

It should be noted in this regard that most investigations on the mode of protein binding of bilirubin were carried out either with icteric serum or with serum to which bilirubin had been added the finding that under these conditions albumin appeared to be the principal carrier does not necessarily indicate that the same protein fraction is responsible for the transport of the much smaller amounts of bilirubin which are present under *physiologic* conditions Indeed Cohn s observation [75] may suggest that the $\alpha_1$ globulin fraction perhaps contains a specific bilirubin binding protein

In healthy individuals the serum bilirubin concentration ranges from 0 1 to approximately 1 5 mg per 100 ml [76] but most values lie between 0 3 and 1 0 mg per 100 ml [14] Many animals such as rats mice dogs and cats have much lower values [14] while in horses the concentration is higher than in man

For excretion in the bile bilirubin is converted to water soluble conjugates [15–17] (Fig 8 5) In man [15–17] cat [77] rat [78] guinea pig [79] dog [17] and sheep [80], most of the bilirubin in the bile is present as diglucuronide while a smaller fraction is excreted as monoglucuronide [81] In addition a minor pigment fraction is present as sulfate [77] Human bile contains trace amounts of other water soluble forms of bilirubin the nature of which is not yet known [77]

Glucuronide formation is the major pathway by which bilirubin is rendered water soluble and suitable for excretion [77 81 82] The glu

Bio ynthesis of bilirubin glucuronide in vitro has been demonstrated with slices [90] homogenates [90 91] and microsomal preparations [84] of rat liver Rat liver preparations also catalyze formation of bilirubin sulfate [77] In addition to liver other tissues have the ability to form glucuronides in vitro particularly the kidney [92] and the mucosa of the upper gastrointestinal tract [92] Nevertheless in vivo extrahepatic conjugation of bilirubin is probably of little physiologic significance although Bollman has suggested that in hepatectomized animals bili rubin monoglucuronide may be formed in organs other than the liver [93]

## THE PATHOPHYSIOLOGY OF HYPERBILIRUBINEMIA

In a general way hyperbilirubinemia occurs as the result of two un related but frequently associated disturbances namely retention in

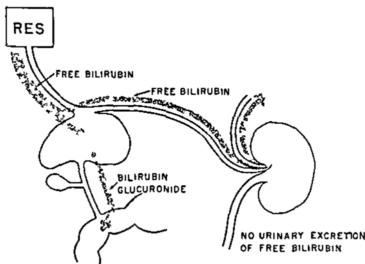


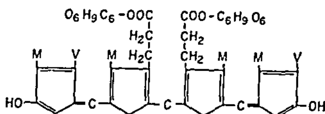

Fig 8-8 In hemolyti jaun li tle pigm nt loa l exceeds the capacity of the liver to l andl l ilirubin

the plasma of *unconjugated* pigment and regurgitation into the plasma of bilirubin which has already been *conjugated* The former group includes the hemolytic syndromes and tho e instances of jaundice where metabolic defects prevent adequate conjugation of bilirubin The second group compri es the *forms of icterus as ociated with extrahepatic obstruction* of the bile ducts and with intrahepatic (functional) cholesta is

In hemolytic jaundice the accelerated rate of red cell de truction leads to overproduction of bilirubin in amounts which may exceed the capacity of the liver to handle the pigment (Fig 8-8) [81 82] It has been shown however that under experimental conditions the ability of normal man [14] or rat liver [94] to conjugate and excrete injected bili

CONJUGATED BILIRUBIN

"DIRECT REACTING'




FREE BILIRUBIN

"INDIRECT-REACTING

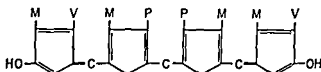



Fig 8-6 In conjugated bilirubin glucuronic acid is attached to the propionic acid radicals of bilirubin to form an acyl glucuronide

ENZYMATIC FORMATION OF BILIRUBIN DIGLUCURONIDE

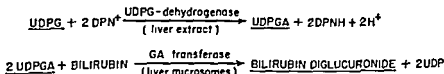



Fig 8-7 Glucuronic acid is transferred from the nucleotide uridine diphosphate glucuronic acid (UDPGA) to bilirubin UDPGA is formed by dehydrogenation of uridine diphosphate glucose (UDPG)

soluble enzyme system which is present in liver [85 87] (Fig 8-7) Glucuronic acid in its *free* form cannot be incorporated into glucuronides but there is suggestive evidence at least in vitro that UDPGA may be formed from uridine triphosphate and glucuronic acid 1 phosphate in the presence of ATP [89]

## NEONATAL HYPERBILIRUBINEMIA

In the liver of fetal mice [97] rabbits [98] rats [99] and guinea pigs [79 92] the rate of formation of glucuronides is much reduced as compared with that of adult animals The same is true of the fetal kidney [92] but apparently not of the gastrointestinal mucosa [92] This reduction appears to involve both synthesis of uridine diphosphate glucuronic acid and activity of the glucuronyl transferase system in the microsomes [79 92] Towards the end of the gestational period fetal tissues exhibit a slow increase in the rate of glucuronide formation but at birth the enzymatic activity still is severalfold below that of adult animals (Fig 8 9)

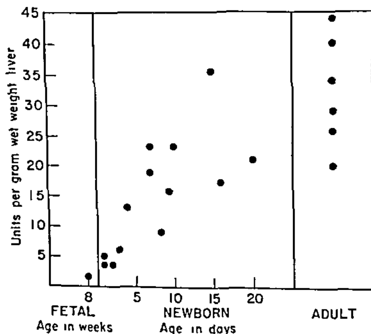



Fig 8-9 Glucuronide formation in fetal newborn and adult liver Formation of o-aminophenyl glucuronide was estimated in liver homogenate of fetal newborn and adult guinea pigs Similar results were obtained with liver homogenate of other mammals (By permission of [illegible])

During the immediate neonatal period the magnitude of glucuronide formation increases rapidly it reaches adult levels a few days to 2 weeks after birth [79 92 98] Attempts to induce increased enzymatic activity in fetal liver by supplying the pregnant rat with large amounts of an aglyconic substrate were unsuccessful even though the substrate passed the placental barrier and accumulated in the fetal liver [100] (Table 8-1) Similar observations have been made with other microsomal enzyme

rubin is so great that in all but the most severe instances of hemolysis it is difficult to understand how increased breakdown of hemoglobin could be the sole cause for the observed retention of pigment in the plasma. This discrepancy has been explained by assuming the coexistence of a relatively specific derangement in liver function. It is also possible that in instances of chronic hemolysis the removal of bilirubin from the plasma may be limited by the rate at which the pigment can be delivered to the liver cells; such a limitation could result from alterations in the plasma protein fractions responsible for the transport of bilirubin.

The icteric syndromes associated with retention of unconjugated bilirubin in the *absence* of significant hemolysis are discussed below.

In acute extrahepatic obstruction of the bile ducts, the predominant pigment in the plasma is bilirubin diglucuronide [93, 95], but smaller increases in monoglucuronide and in unconjugated bilirubin are often also present [93, 95]. In chronic obstruction, bilirubin monoglucuronide is frequently found in larger amounts than diglucuronide or unconjugated bilirubin [93, 95]. This is believed to be due to variable degrees of hepatic cell dysfunction in addition to the obstruction [81].

In hepatitis and in cirrhosis the plasma usually contains both conjugated and unconjugated bilirubin [81, 82]. Thus, in addition to the impairment in hepatocellular function, these conditions are associated with a defect in excretory function, but this explanation is not entirely satisfactory. Future studies on the fine structure of the liver, and particularly on the mechanisms involved in the excretion of the various bile constituents, may reveal information requiring considerable modification of present concepts.

## HYPERBILIRUBINEMIA ASSOCIATED WITH DEFECTIVE FORMATION OF CONJUGATED BILIRUBIN

In human bile and in that of other mammals essentially all bilirubin is present in conjugated form, mostly as mono- or diglucuronide. Conjugation is probably essential for the excretion of bilirubin in the bile [78]. Thus it would be expected that impaired formation of conjugated bilirubin results in reduced pigment excretion and, hence, in retention of unconjugated bilirubin in the plasma. As mentioned earlier, such a defect may involve the transport of bilirubin into the liver cells, the penetration of the pigment into the subcellular microsomes, or the activity of the microsomal enzyme system catalyzing the actual conjugation.

A transient type of hyperbilirubinemia, probably due to reduced activity of the glucuronide forming enzyme system, is frequently observed during the first days of the neonatal period [96]. Hereditary forms of icterus associated with similar enzymatic defects are found in man [64, 66] and in the rat [78].

this pathway and results in transient hyperbilirubinemia which persists until the neonatal liver reaches the functional capacity to handle its own pigment load

## HEREDITARY HYPERBILIRUBINEMIA IN RATS (GUNN STRAIN)

Gunn [102] and Malloy and Loewenstein [103] described a mutant strain of Wistar rats exhibiting hereditary acholuric jaundice The disturbance is probably inherited as a Mendelian recessive trait since litters produced by nonicteric parents known to be carriers of the trait

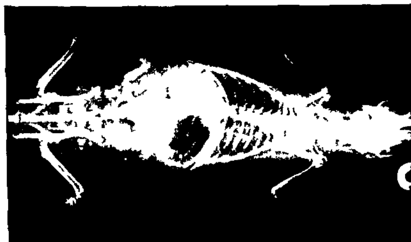


Fig 8-10 Biliary excretion of Cholografin in rat with hereditary hyperbilirubinemia The contrast medium was demonstrable by x ray in the small bowel 20 min after its intravenous injection Rats lack a gallbladder

usually include 25 per cent icteric offspring [102] Icteric animals exhibit bilirubin levels ranging from approximately 5 mg per 100 ml to well above 20 mg All plasma bilirubin is of the unconjugated type and bilirubinuria is absent [78] There is no evidence of increased hemolysis [103] and liver morphology is normal [78] In rats with bilirubin level exceeding 12 to 15 mg per 100 ml functional impairment and morphologic alterations of the central nervous system resembling kernicterus are frequently observed [104]

The biliary excretory system is patent as demonstrated by rapid excretion of radiopaque contrast medium (Cholografin) and of Bromsulphalein [78] (Fig 8 10) Moreover conjugated bilirubin injected intravenously is rapidly and completely excreted in the bile with biliary pigment concentration rising one hundred fold (Fig 8 11) [78] Maximal clearance for *conjugated* bilirubin in *jaundiced* rats is similar to the maximal clearance for *unconjugated* bilirubin in *normal* rats as determined

systems which are "underdeveloped in the fetus and reach their full activity only after birth [101]

In man estimates of the magnitude of glucuronide formation in fetal and newborn liver are difficult to obtain Dutton [92] studied the liver of two human fetuses aged 3 to 4 months and found very low activity of both glucuronyl transferase and uridine diphosphate glucose dehydrogenase In the liver of three prematurely born infants (birth weight from 766 to 1 312 gm), Lathe and Walker observed only negligible glucuronide formation [90] These two reports together with the more extensive observations in other mammalian species, suggest that the inadequate development of the glucuronide-forming enzyme system at

TABLE 8-1 BIOSYNTHESIS OF o-AMINOPHENYL GLUCOSIDURONIC ACID BY MICROSOMES OF MATERNAL AND FETAL RAT LIVER AFTER REPEATED INJECTION OF o-AMINOBENZOATE TO THE MOTHER

| Type of treatment | No of animals | o-Aminophenyl glucosiduronic acid formed μmoles/gm | |
|---|---|---|---|
| | | Mother | Fetus |
| o-Aminobenzoate | 6 | 0 011 ± 0 013 | 0 008 ± 0 00? |
| Saline controls | 6 | 0 011 ± 0 019 | 0 011 ± 0 003 |

SOURCE R Schmid et al [100]

birth is probably the most important factor in producing the transient hyperbilirubinemia in the newborn In infants with erythroblastosis fetalis the increased pigment load resulting from the hemolytic process is superimposed upon this functional defect in the liver

The fate of the bilirubin produced by the fetal organism during the *prenatal* period has been a matter of great interest Since the fetal conjugating apparatus is operating at a low level of efficiency significant excretion of bile pigment into the intestinal tract cannot occur On the other hand the bilirubin level of fetal blood is usually low but it rises steeply after severance of the umbilical cord [96] These observations point to the placenta as the organ responsible for clearing the fetal blood of excess bilirubin Until recently it was not known whether the fetal pigment is conjugated *in the placenta or whether it is transferred to the* maternal circulation for conjugation in the maternal liver In human and rat placenta no evidence for the presence of a functioning glucuronide-forming mechanism was obtained [100] On the other hand it was found that in guinea pigs unconjugated bilirubin can be rapidly transferred from fetal blood through the placenta to the maternal circulation [100] These observations suggest that bilirubin produced in the fetus may reach the maternal blood unchanged and may then be conjugated and excreted by the maternal liver Severance of the umbilical cord interrupts

this pathway and results in transient hyperbilirubinemia which persists until the neonatal liver reaches the functional capacity to handle its own pigment load

HEREDITARY HYPERBILIRUBINEMIA IN RATS (GUNN STRAIN)

Gunn [*102*] and Malloy and Loewenstein [*103*] described a mutant strain of Wistar rats exhibiting hereditary acholuric jaundice The disturbance is probably inherited as a Mendelian recessive trait since litters produced by nonicteric parents known to be carriers of the trait

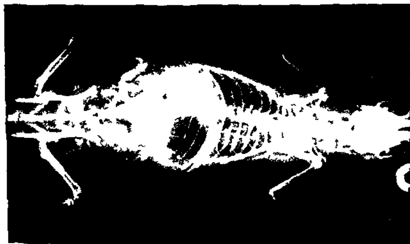


Fig 8-10 Biliary excretion of Cholografin in rat with hereditary hyperbilirubinemia The contrast medium was demonstrable by x ray in the small bowel 20 min after its intravenous injection Rats lack a gallbladder

usually include 25 per cent icteric offspring [*102*] Icteric animals exhibit bilirubin levels ranging from approximately 5 mg per 100 ml to well above 20 mg All plasma bilirubin is of the unconjugated type and bilirubinuria is absent [*78*] There is no evidence of increased hemolysis [*103*] and liver morphology is normal [*78*] In rats with bilirubin levels exceeding 12 to 15 mg per 100 ml functional impairment and morphologic alterations of the central nervous system resembling kernicterus are frequently observed [*104*]

The biliary excretory system is patent as demonstrated by rapid excretion of radiopaque contrast medium (Cholografin) and of Bromsulphalein [*78*] (Fig 8 10) Moreover conjugated bilirubin injected intravenously is rapidly and completely excreted in the bile with biliary pigment concentration rising one hundred fold (Fig 8 11) [*78*] Maximal clearance for *conjugated* bilirubin in *jaundiced* rats is similar to the maximal clearance for *unconjugated* bilirubin in *normal* rats as determined

by Weinbren and Billing [94] In icteric rats on the other hand intravenous administration of *unconjugated* bilirubin results in biliary excretion of less than 1 per cent of the amount excreted by normal rats with a comparable serum bilirubin level [78]

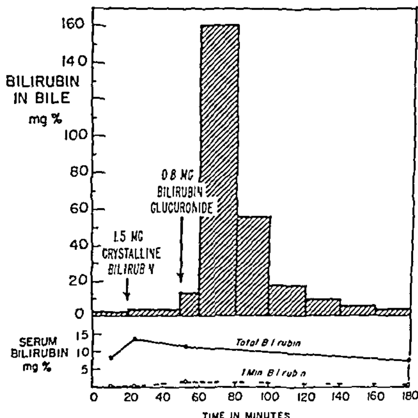



Fig 8-11 Biliary excretion of injected bilirubin in rat with hereditary hyperbilirubinemia Crystalline bilirubin and conjugated bilirubin were injected in a tail vein of a jaundiced rat The conjugated pigment was rapidly excreted in the bile while injection of crystalline bilirubin resulted only in a slightly increased concentration of nonconjugated bilirubin (*By permission of R Schmid et al [78]*)

The bile of icteric rats contains much less bilirubin than that of animals without jaundice and the small amount of pigment present is in the *nonglucuronide* form [78] Furthermore fecal urobilinogen excretion in jaundiced rats is greatly reduced as compared with that in control animal [78] (Table 8-2) but the color of the feces is normal

Total urinary excretion of glucuronic acid and glucuronides is approximately half of that in control animals [78] When compounds that are normally excreted as glucuronides are administered to icteric rats the rise in urinary *glucuronide excretion is significantly* smaller than in con

TABLE 8-9 FECAL UROBILINOGEN EXCRETION IN WISTAR RATS

| Rats | No of animals | Urobilinogen mg/day/100 gm body weight | |
|---|---|---|---|
| | | Mean | Standard deviation |
| Jaundiced (Gunn strain) | 3 | 0 003 | ±0 004 |
| Nonjaundiced litter mates (Gunn strain) | 3 | 0 039 | ±0 013 |
| Normal control | 4 | 0 077 | ±0 0[illegible]4 |

SOURCE R Schmid et al [78]

trol animals [78] (Fig 8 12) This is observed both with aglycones that form ethereal glucuronides such as menthol and with ester forming aglycones such as o-aminobenzoic acid [78] Likewise injection of benzoate to jaundiced rats results in urinary excretion of larger amounts of hippurate and smaller amounts of benzoyl glucuronide as compared with excretion of these substances in nonicteric animals [78]

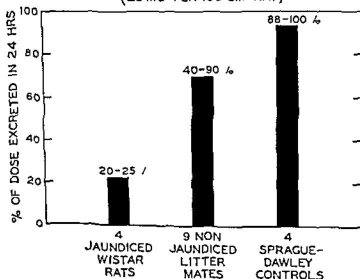



Fig 8-12 Urinary excretion of o-aminobenzoyl glucuronide was estimated after intraperitoneal injection of a loading dose of o-aminobenzoate (anthranilate) In icteric rats a much smaller fraction of the administered compound was excreted in the form of the glucuronide as compared with normal rats Nonjaundiced littermates of icteric animals excreted less glucuronide than genetically normal rats but more than icteric rats (*By permission of R Schmid et al [78]*)

Glucuronide formation with liver of jaundiced rats was studied in vitro by incubating slices [90] homogenates [90, 105] and microsomal preparations [50, 78]. In all instances, glucuronide synthesis was reduced but the results differed depending on the substrate used for the enzymatic assay. With bilirubin as substrate formation of bilirubin glucuronide could not be detected [105] whereas with o-aminophenol o-aminophenyl glucuronide was formed but at a greatly reduced rate [78, 90] (Table 8-3)

TABLE 8-3 ENZYMATIC SYNTHESIS OF URIDINE DIPHOSPHATE GLUCURONIC ACID (UDPG) AND OF o-AMINOPHENYL GLUCOSIDURONIC ACID BY MALE RAT LIVER MICROSOMES

| *Rats* | *No. of animals* | *UDPG* | *o-Aminophenyl glucosiduronic acid* |
|---|---|---|---|
| Jaundiced | 6 | 4.9 | 0.008 |
| Nonjaundiced litter mates | 8 | 4.3 | 0.034 |
| Normal controls | 7 | 5.8 | 0.061 |

Results expressed in micromoles formed per gram of liver
SOURCE R. Schmid et al. [78]

In jaundiced rats the activity of uridine diphosphate glucose dehydrogenase in the liver was comparable to that in normal animals [78]. Addition of excess uridine diphosphate glucuronic acid to microsomal preparations of icteric rat liver failed to augment glucuronide synthesis [78, 90]. In kidneys of icteric rats, a comparable reduction in glucuronide formation was observed [78, 105].

These findings suggest that the primary defect in jaundiced rats involves the glucuronyl transferase system in the microsomes. It remains unexplained, however, why glucuronide formation, both *in vivo* and *in vitro*, is not demonstrable with bilirubin whereas with other compounds small but significant amounts of the glucuronides are formed [78, 90]. It has been suggested that bilirubin may be unsuccessful in competing with other aglycones for the deficient glucuronide-forming enzyme system [55], but this explanation is difficult to reconcile with the observation that injection of o-aminobenzoate *does* result in increased glucuronide excretion [though the increase is smaller than in nonicteric animals (Fig. 8-12)] whereas bilirubin has no such stimulatory effect. Another possible explanation is that the formation of different types of glucuronides is catalyzed by separate microsomal enzyme systems and that in jaundiced rats only the enzyme involved in the synthesis of bilirubin glucuronide is deficient. However, this is not borne out by the experimental findings in that formation of glucuronides is reduced with *all* aglyconic compounds that have been tested.

An attempt was made to stimulate the activity of the glucuronide-forming enzyme system in jaundiced rats by repeated administration of

large amounts of an aglyconic substrate other than bilirubin [80] For 5 consecutive days 50 mg per 100 gm body weight of sodium o-aminobenzoate was injected twice a day by intraperitoneal route At the end of this period liver microsomes prepared from these animals failed to exhibit increased activity of glucuronyl transferase as compared with similar preparations from icteric rats injected with equivalent amounts of sodium chloride In the icteric rats treated with o aminobenzoate the liver exhibited considerable concentrations of the administered compound as long as 20 hr after the last injection whereas in nonicteric control animals the injected o-aminobenzoate was conjugated and removed from the liver within a few hours [80] These findings indicate that the defect in glucuronyl transferase activity cannot be corrected by supplying excessive amounts of substrate This conclusion could be anticipated because the increased concentration of circulating bilirubin would be expected to provide an already adequate stimulation for enzyme induction

From the above discussion it is apparent that the hyperbilirubinemia in this strain of rats results from an enzymatic defect which precludes conversion of bilirubin to its water soluble glucuronide and hence interferes with the excretion of the pigment in the bile A defect of this type had already been anticipated by Malloy and Loewenstein [103]

It is of interest that in spite of this severe impairment in pigment excretion the physiologic turnover of heme compounds does not result in a steadily increasing degree of jaundice It must be concluded that alternate pathways for heme or bilirubin catabolism must exist [78] which at these increased bilirubin levels are capable of establishing a new balance between formation and breakdown of pigment

In nonicteric rats which are known carriers of the jaundice trait glucuronide formation in vivo and in vitro is reduced as compared with that in genetically normal rats but is greater than that in icteric rats [78 90] Thus in animals which are heterozygous for the trait the metabolic abnormality is present in a less severe degree and is insufficient to give rise to pigment retention in the plasma

This strain of icteric rats provides an unusual opportunity for the study of bilirubin metabolism and they are an excellent experimental tool for investigations into the pathogenesis of kernicterus [104]

## CONGENITAL FAMILIAL NONHEMOLYTIC JAUNDICE IN MAN (CRIGLER NAJJAR SYNDROME)

### *Clinical Findings*

A familial form of severe nonhemolytic jaundice associated with disturbances of the central nervous system was described in 1952 by Crigler and Najjar [63] In three related families a total of six infants was ob-

served in whom severe icterus appeared on the first or second day after birth and persisted throughout lifetime. There was no evidence of increased hemolysis or of blood group incompatibilities. The serum bilirubin was virtually all of the indirect reacting type and bilirubinuria was absent. Liver histology and conventional liver function tests failed to reveal significant abnormalities.

All but one of the icteric infants developed severe disturbances of the central nervous system resembling kernicterus, and all five died during the first 15 months of life. In one instance where the brain could be examined at autopsy the cerebral cortex and the basal ganglia showed intense staining with bile pigment. The sixth icteric child of this family group failed to develop neurologic abnormalities [106] and at the time of this writing is 8 years old and well except for persistent jaundice. It is not known why only one of the six affected children escaped neurologic damage, but it may be significant that during the first months of life *this child's serum bilirubin level was somewhat lower than those of the* other infants in whom serial determinations were reported [53].

An additional case belonging to the same family group was discovered in 1956 [106]. This now 5 year old girl, a double first cousin of the other patients, developed icterus on the first or second day of life. Serum bilirubin levels of approximately 25 mg per 100 ml have persisted since birth but neurologic disturbances have so far not been detected [106].

A few other cases exhibiting similar features have been observed and in part reported in the literature. Rosenthal et al. [107] studied a 5 year old boy who became icteric on the second day of life. The serum bilirubin concentration has remained between 20 to 35 mg per 100 ml and almost all of it gives an indirect van den Bergh reaction. Bilirubinuria was never present. Despite the persistent icterus the child remained well until the age of 3 when slight slurring of speech and intention tremor were noticed. At the age of 5 years, in the course of a few weeks, severe disturbances of motor function developed with ataxia and severe dysarthria. When last seen, at the age of 9, the boy was severely crippled.

Both parents and a younger brother of this patient have normal bilirubin levels [107]. A striking feature of the family history is that the mother's brother and the father's sister, who are married to each other, had a first child who died at the age of 4 weeks with jaundice and central nervous system disturbances. At necropsy no evidence for hemolytic or hepatobiliary disease could be detected; unfortunately, the brain was not examined.

The only patient with this syndrome believed to have reached adult life was reported by Jervis [108]. This woman, who lived the last 30 years of her life in a mental institution, died from pneumonia at the age of 44. At the age of 6 months the patient was diagnosed as having "Little's disease with idiocy and jaundice." On admission to the mental institution

he exhibited deep jaundice severe muscular rigidity dystonia partial deafness and mental deficiency The e manifestations did not progress but the bilirubin level remained between 16 to 22 mg per 100 ml At autopsy the liver was found to be grossly and histologically normal but the brain was reduced in weight and on histologic examination exhibited degenerative changes consistent with kernicterus

Klingberg et al [109] observed two siblings with persistent severe icterus and central nervous system manifestations both of whom died during the first year of life At autopsy no evidence for increased hemolysis liver di ea e or biliary obstruction could be detected

TABLE 8-4 CLINICAL AND LABORATORY FINDINGS IN PATIENTS WITH CONGENITAL NONHEMOLYTIC JAUNDICE

| *Patient and reference* | *Sex* | *Age icterus noted* | *Total serum bilirubin (range mg/100 ml)* | *Evidence of neurologic involvement* | *Condition in 1955* |
|---|---|---|---|---|---|
| I M [ 3] | F | 2 days | 28-37 | Clinical | Died at 21 wk |
| J R H [ 3] | M | 2 days | 26 | Clinical and anatomic | Died at 18 days |
| J D H [100] | M | 3 days | 17 27 | None | Living at 8 yr |
| J I T [ 3] | M | 2 days | 10-33 | Clinical | Died at 16 mo |
| L T [53] | M | 2 days | 13-45 | Clinical | Died at 20 wk |
| J J T [53] | M | Birth | Icterus index 50 | Clinical | Died at 47 wk |
| M L H [100] | F | 1 day | 25 | None | Living at 5 yr |
| J D [10 ] | M | 2 days | 20-35 | Clinical | Living at 9 yr |
| [108] | F | 6 mo | 16 22 | Clinical and anatomic | Died at 44 yr |
| J d F [80] | M | 3 days | 20-25 | None | Living at 2½ yr |
| R S [80] | F | 1 day | 16– 0 | None | Living at 8 yr |
| W J H [109] | M | 3 days | 15–35 | Clinical and anatomic | Died at 1 yr |
| J H [109] | F | 3 days | 29 | Clinical and anatomic | Died at 1 yr |

Early observation not reported [108]

In addition to the e case the author is following two patients with severe persistent jaundice Both are similar to those described above except that to date they have escaped detectable damage to the central nervous system [1] The older (R S) a girl of 8 with a bilirubin concentration between 16 to 20 mg per 100 ml underwent two exploratory laparotomies which revealed normal liver histology and patency of the biliary tree The younger is a boy of two (J d E) whose bilirubin concentration ranges from 20 to 25 mg per 100 ml These cases are summarized in Table 8-4

[1] The author is indebted to Drs I K D n on and S Cell Ik ton f r permission to study these patients

*Laboratory Examinations*

In none of the cases patients was there evidence of hemolytic anemia as shown by normal hemoglobin concentration low reticulocyte counts, normal bone marrow morphology and absence of splenomegaly The liver was not enlarged and on histologic examination, the only abnormality *found was the presence of bile thrombi in hepatic canaliculi in*



Fig 8-13 Visualization of the gallbladder in patients with congenital nonhemolytic jaundice *In a patient with congenital nonhemolytic jaundice exhibiting a serum* bilirubin concentration of 35 mg per 100 ml intravenous cholangiography showed normal visualization of the gallbladder (*Courtesy of Dr I M Rosenthal Chicago*)

*some of the cases [53]* *Conventional liver function tests* including clearance of Bromsulphalein yielded uniformly normal results In one child a low value for urinary excretion of hippuric acid was observed after oral administration of sodium benzoate [*107*] when this test was later *repeated with intravenous sodium benzoate hippurate excretion in the* urine was within normal limits [*80*]

In two cases intravenous cholangiograms [*80* *107*] showed normal visualization of the gallbladder and the biliary ducts (Fig 8 13) In two patients patency of the extrahepatic bile ducts was established at laparatomy

The urine contained no bilirubin as shown by negative Gmelin Fouchet, and van den Bergh tests In the four cases studied by the author the urine was of a golden yellow color which significantly differed in shade and intensity from that of normal urine The nature of these yellow pigments is not known but they exhibited some similarities to those present in the plasma and urine of hepatectomized dog [110]

Bile was obtained from the duodenum of four children before and after stimulation of bile flow with Decholin or magnesium sulfate [54 80]

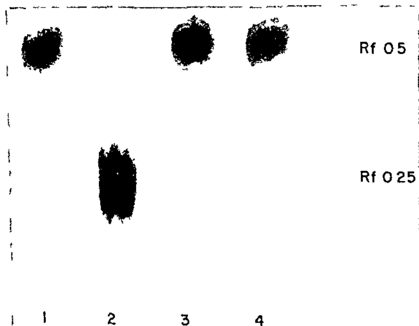



Fig 8-14 Bilirubin in serum of patients with congenital nonhemolytic jaundice Ascending paper chromatogram of the azoderivatives of serum bilirubin from three patients with congenital nonhemolytic jaundice All three serums contain only non conjugated bilirubin ($R_F$ 0 5) The azoderivative of bilirubin diglucuronide has an $R_F$ of 0 25 (1) Case J D mal (2) Control bilirubin glucuronate (3) Case J H female (4) Case J D H male (*By permission of R Schmid [54]*)

In three the bile specimens had a faint lemon juice color and bilirubin concentrations ranged from 0 1 to 1 5 mg per 100 ml Essentially all of the pigment was present in unconjugated form In all three fecal urobilinogen excretion was greatly reduced ranging from 0 23 mg per 24 hr (2 years old) to 5 mg per 24 hr (7 years old) while serum bilirubin concentrations were in excess of 20 mg per 100 ml

In the fourth child (R S ) with a serum bilirubin of 17 mg per 100 ml the duodenal bile contained 4 mg bilirubin per 100 ml and about half was in the form of the glucuronide This was later confirmed on lap-

*Laboratory Examinations*

In none of these patients was there evidence of hemolytic anemia as shown by normal hemoglobin concentration low reticulocyte counts normal bone marrow morphology, and absence of splenomegaly The liver was not enlarged and on histologic examination, the only abnormality found was the presence of bile thrombi in hepatic canaliculi in



Fig 8 13 Visualization of the gallbladder in patients with congenital nonhemolytic jaundice In a patient with congenital nonhemolytic jaundice exhibiting a serum bilirubin concentration of 35 mg per 100 ml intravenous cholangiography showed normal visualization of the gallbladder (*Courtesy of Dr I M Rosenthal Chicago*)

some of the cases [*53*] Conventional liver function tests including clearance of Bromsulphalein yielded uniformly normal results In one child a low value for urinary excretion of hippuric acid was observed after oral administration of sodium benzoate [*107*] when this test was later repeated with intravenous sodium benzoate hippurate excretion in the urine was within normal limits [*80*]

In two cases intravenous cholangiograms [*80 107*] showed normal visualization of the gallbladder and the biliary ducts (Fig 8 13) In two patients patency of the extrahepatic bile ducts was established at laparatomy

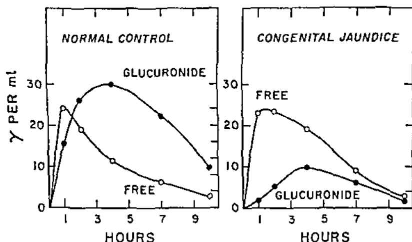



Fig 8-15 A male child with congenital nonhemolytic jaundice and a control subject of the same age and sex were given an oral dose of 30 mg per kg N acetyl *p*-amino phenol (NAPA) The concentration of NAPA glucuronide in the plasma of the icteric patient was much smaller than in the normal control (*By p rmi sion of J Axelrod et al* [55])

a partial deficiency in the conjugation of reduced steroids with glu curonic acid

Similar results were obtained after oral administration of menthol [54] chloral hydrate [113] trichlorethanol [113] and salicylate [80 113] and after intravenous injection of NAPA [80] Following injection of NAPA for example the total recovery of NAPA metabolites in the

TABLE 8-5 URINARY EXCRETION OF N ACETYL-p-AMINOPHENYL (NAPA) GLUCURONIDE AND TOTAL p-AMINOPHENOL IN THREE PATIENTS WITH CONGENITAL NONHEMOLYTIC JAUNDICE AFTER A SINGLE INTRAVENOUS INJECTION OF NAPA 10 MG PER KG

| *Patient age and sex* | *Patient* | | *Controls of same age and sex* | |
|---|---|---|---|---|
| | *NAPA glucuronide* | *Total p-aminophenol* | *NAPA glucuronide* | *Total p-aminophenol* |
| J d E (8 months male) | 8 9 | 50 | 25 | 71 |
| | | | 28 | 58 |
| J D (8 years male) | 16 6 | 48 | 30 | 51 |
| R S (8 years female) | 18 0 | 65 | 25 | 55 |
| | | | 29 | 54 |

Amounts excreted in 6 hr in per cent of dose administered

anatomy when bile aspirated from the gallbladder was found to contain 70 mg bilirubin per 100 ml about one third conjugated with glucuronic acid [80] Fecal urobilinogen excretion in this 8 year-old girl was 16 9 mg per 24 hr The lack of reliable data on fecal pigment excretion by healthy children of this age group makes it impossible at present to determine whether this represents a significant reduction in fecal urobilinogen excretion It is of interest that in contrast to extrahepatic biliary obstruction the color of the feces of all four icteric children was normal [80]

In the original report of this syndrome [53] it was stated that fecal *urobilin* excretion was normal but these results were obtained by a method not in common use [111] For instance in a 6-year-old boy originally reported as having normal *urobilin* excretion [53] fecal *urobilinogen* excretion was found to be 1 4 mg per 24 hr [80]

In all patients studied the serum was deeply icteric Bilirubin concentrations ranged from 16 to 38 mg per 100 ml and practically all of it gave an indirect van den Bergh reaction On paper chromatographic analysis no evidence for the presence of bilirubin glucuronide was obtained [10 54] (Fig 8 14) In some instances the plasma was extracted and bilirubin was prepared in crystalline form [53]

*Formation of Glucuronides in Vivo and in Vitro*

The above findings suggest that the metabolic defect in these patients involves the conversion of bilirubin to its water soluble conjugates [54 55] Direct demonstration of such a disturbance in vivo is technically difficult but it was found that in addition to impaired formation of bilirubin glucuronide formation of other glucuronides is also reduced [54 55] This was demonstrated with a variety of compounds which ordinarily are excreted in part as glucuronides

After oral administration of N acetyl *p*-aminophenol (NAPA), the concentration of NAPA glucuronide in the plasma was much less in an icteric child than in a normal subject of the same age and sex [55] (Fig 8 15) When tetrahydrocortisone or tetrahydrocortisol was given by rapid intravenous infusion the rate of disappearance of these compound from the plasma of icteric children was slower than in normal subjects [112 113] (Fig 8-16) A difference in disappearance rate was not noted with cortisol[2] [112 113] but the metabolites of cortisol conjugated with glucuronic acid appeared in the plasma at a slower rate than in normal subjects [112] (Fig 8 17) Following infusion of cortisol $C^{14}$ recovery of labeled metabolites in the urine of icteric patients was quantitatively comparable to that in normal subjects but the fraction of glucuronic acid–conjugated metabolites was much smaller and the fraction of other conjugates larger than in normal individuals [112] These findings indicate

[2] Cortisol in contrast to tetrahydrocortisol does not form a glucuronide because it lacks the 3α-ol function in ring A [114]

formation of glucuronides is demonstrable with all test substances used but glucuronide synthesis is by no means absent For bilirubin on the other hand a much more severe defect must be assumed if the pigment retention in the plasma and the impaired excretion of bilirubin in the

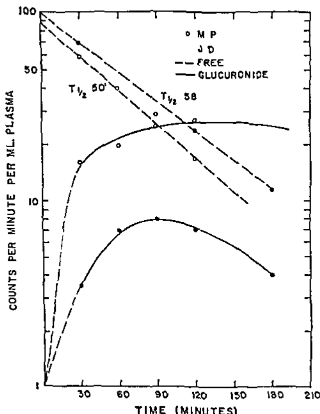



Fig 8-17 The rate of disappearance of injected hydrocortisone (cortisol) was similar in a child with congenital nonhemolytic jaundice (J D ) and in a control subject of the same age and sex (M P ) The rate of appearance of glucuronic acid-conjugated metabolites of cortisol in the plasma was slower in the icteric patient than in the control subject (*Courtesy of R E Peterson and R Schmid unpublished observation*)

bile and of urobilinogen in the feces are due *solely* to a disturbance in the conjugating apparatus The problem is strikingly similar to that in the icteric rats discussed earlier in both the human and the murine syndromes it remains unexplained why the defect affects glucuronide forma

urine of jaundiced children was comparable to that in control subjects but the glucuronic acid conjugated fraction was reduced in the presence of icterus (Table 8 5)

Reduced formation of glucuronides has also been reported with salicylate [118] but interpretation of the results is complicated because

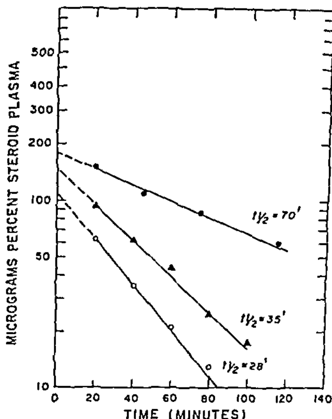



Fig 8-16 Disappearance of tetrahydrohydrocortisone (tetrahydrocortisol) from plasma following intravenous administration ● Patient with congenital nonhemolytic jaundice ▲ patients (3) with parenchymal liver disease ○ normal subjects (4) (*By permission of K F Peterson et al [112]*)

normal individuals excrete only 10 to 15 per cent of the administered dose as glucuronides and because salicylate forms both phenolic and acyl glucuronides [115] In two icteric children given sodium salicylate by mouth the urinary excretion of salicyl glucuronides *and* the total excretion of salicylate metabolites was reduced [113] In the glucuronide fraction, the reduction was more pronounced for the phenolic type of glucuronide than for the acyl type [113] which is surprising since bilirubin forms an acyl glucuronide [83]

These findings do not permit a final interpretation A reduction in the

infancy with severe unexplained jaundice These observations suggest that the defect is genetically determined and may be inherited as a Mendelian recessive trait Unfortunately the available reports do not permit a calculation of the ratio of affected children to unaffected siblings

In parents and nonaffected siblings of icteric patients serum bilirubin concentrations were consistently normal [106 107] The magnitude of glucuronide formation in vivo was studied in parents and siblings of six icteric patients [80 113] It should be noted that the significance of such

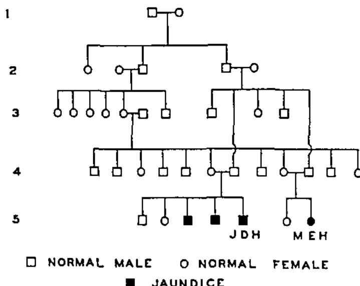



Fig 8-18 Pedigree of a family with congenital nonhemolytic jaundice This family is part of an interrelated family group exhibiting a total of seven recognized cases of jaundice (*By permission of B Childs et al* [113])

family studies is limited by the existing uncertainty with regard to the exact pathogenesis of the syndrome and by the fact that even in patients with jaundice glucuronide formation is only moderately reduced

Childs et al [113] fed sodium salicylate to parents adult siblings and grandparents of three icteric patients and estimated the excretion of salicyl glucuronides and of other salicylate metabolites in the urine In the majority but not in all of the parents a slight reduction in excretion of glucuronides was demonstrable [113] The mean value for salicyl glucuronide excretion by parents adult siblings and grandparents combined was moderately reduced in two families and questionably reduced in the third These findings were believed to indicate that the abnormal gene is recessive with respect to bilirubin but incompletely dominant for other glucuronide-forming compounds [113]

tion with bilirubin more severely than with other glucuronide-forming compounds

Studies of liver tissue in vitro in this syndrome have not been reported except in two patients who originally were believed to have Gilbert s disease [116] but who probably had a defect similar to that under discussion [117] In these two patients liver homogenate was found to lack the ability to form glucuronides both with bilirubin and with o-aminophenol [116] but in one instance the bile contained "direct reacting bilirubin" although chromatographic analysis of the pigment was not attempted [116]

In the author s laboratory liver tissue was studied from the 8 year-old girl (R S ) who exhibited serum bilirubin levels of 16 to 20 mg per 100 ml Bilirubin and o-aminophenol were incubated with liver homogenate and with a microsomal preparation respectively It was observed that liver homogenate failed to form direct reacting bilirubin [90] but the microsomal preparation synthesized o-aminophenyl glucuronide at a rate which was about half of that found with similar preparations of male rat liver [78] In addition however bile aspirated from the gall bladder contained 70 mg per 100 ml bilirubin of which about one-third was in the form of the glucuronide [118] and less than 5 per cent in the form of the alkali stable sulfate [77] This observation must indicate that formation of bilirubin glucuronide *did* occur in vivo but with the available methods it could not be demonstrated in vitro A definite explanation for this perplexing finding cannot be offered The possibility should be considered however that the defect in glucuronide formation may be associated with other abnormalities in bilirubin metabolism perhaps involving the binding of pigment to plasma proteins or bilirubin transport into the liver cells

The available evidence relating to the pathogenesis of congenital non hemolytic jaundice may be summarized as follows marked retention of unconjugated bilirubin occurs in the plasma and biliary excretion of bilirubin is reduced This is the result of a disturbance in the liver involving conversion of bilirubin to its water soluble conjugates The precise nature of this defect is not yet clear and it is not known whether it involves only reduced activity of the glucuronyl transferase system in the microsomes or includes other aspects of bilirubin metabolism in the liver

### *Genetic Considerations*

The original description of this syndrome [58] indicated a definite familial occurrence Six cases occurred in three related families and later an additional genetically related patient was discovered (Fig 8 18) [106] The two patients observed by Klingberg [109] were siblings and a double cousin of the patient reported by Rosenthal et al [107] died in

examination [120] The bilirubin level frequently shows considerable daily fluctuation Among 58 patients followed for various lengths of time 13 had levels of 1 mg per 100 ml or less at least at some time during the observation period [120] In this group the average of the highest values observed in each patient was 2 72 mg per 100 ml and the average of the lowest observed levels 1 67 mg per 100 ml [120] In 81 per cent of the patients the initial concentration was less than 3 mg per 100 ml Virtually all the serum bilirubin gives an indirect van den Bergh reaction [120] and on paper chromatographic analysis no conjugated bilirubin is detectable [80] Bilirubinuria is absent and conventional liver function tests give normal results except for occasional increased Bromsulphalein retention [117, 125] Liver and spleen are usually not enlarged [120 126] and histologic examination of the liver reveals no significant abnormalities [120]

It is often stated that the hyperbilirubinemia is associated with subjective symptoms such as fatigue asthenia weariness and dyspepsia [121 122 126] The multiplicity of complaints and the failure to elicit any common pattern of symptoms suggest that these manifestations may be unrelated to the disorder in pigment metabolism [120] Indeed it seems likely that many of these symptoms develop *after* the patient has become aware of his icterus and are related to anxiety rather than to organic disease [120] For example four young physicians exhibiting serum bilirubin levels of 1 4 to 2 8 mg per 100 ml denied any systemic symptoms whereas two young ex service men whose mild hyperbilirubinemia was detected during active duty entitling them to disability payments complained of excessive fatigue and lassitude [80]

In some of the patients studies of erythrocyte survival with the $Cr^{51}$ method suggested a mild and compensated hemolytic state [120 127] It is doubtful whether this slight decrease in erythrocyte life span could account for the chronic hyperbilirubinemia since as discussed earlier the ability of the normal liver to remove bilirubin from the blood stream is much in excess of the pigment load presented by the physiologic decay of erythrocytes The fecal excretion of urobilinogen is usually normal or may be slightly decreased

The pathogenesis of this syndrome is not clear and there is much evidence to suggest that persistent mild hyperbilirubinemia may be the result of several unrelated disturbances Hult [126] and Volwiler and Elliott [128] showed that chronic icterus may be a late manifestation of infectious hepatitis On the other hand the finding of unexplained mild hyperbilirubinemia in several members of a family [120–123 129] and the occurrence of mild icterus in three successive generations [130] suggest that the abnormality may be genetically controlled Finally it should be considered that some of the individuals with an apparent increase in indirect reacting bilirubin concentration may simply represent

The parents of three other icteric children were studied by intravenous injection of NAPA followed by estimation of the excreted NAPA glucuronide and total aminophenol in the urine [80] As seen in Table 8 6 four out of the six parents tested excreted at least as much NAPA glucuronide as control subjects of the same sex, while in two the values were slightly reduced These findings which are at variance with those

TABLE 8-6 URINARY EXCRETION OF N-ACETYL-p-AMINOPHENYL (NAPA) GLUCURONIDE AND TOTAL p-AMINOPHENOL IN PARENTS OF PATIENTS WITH CONGENITAL NONHEMOLYTIC JAUNDICE AFTER A SINGLE INTRAVENOUS INJECTION OF NAPA 10 MG PER KG

| *Parents* | *NAPA glucuronide* | *Total p-aminophenol* |
|---|---|---|
| J d F | | |
| Mother | 3[illegible] | [illegible] |
| Father | 34 | 54 |
| J D | | |
| Mother | 29 | [illegible] |
| Father | [illegible] | 74 |
| R S | | |
| Mother | 45 | 8[illegible] |
| Father | 36 | 70 |
| Normal controls | | |
| 3 females (mean) | 38 | 68 |
| 3 males (mean) | 32 | 5[illegible] |

*Amounts excreted in 6 hr in per cent of dose administered*

reported by Childs et al [118] failed to reveal a consistent reduction in glucuronide formation in parents of patients with congenital nonhemolytic jaundice They do not support the concept of a dominant inheritance of this metabolic disturbance [118]

## CONSTITUTIONAL HEPATIC DYSFUNCTION (GILBERT'S DISEASE)

Since the original report by Gilbert [119] patients with unexplained mild and chronic elevation of indirect reacting serum bilirubin have been reported with increasing frequency [120] The condition has variously been referred to as icterus intermittens juvenilis [121] hereditary nonhemolytic bilirubinemia [122] familial nonhemolytic jaundice [123] and constitutional hepatic dysfunction [124] None of these terms is adequate but any is perhaps preferable to the frequently employed Gilbert's disease since most of Gilbert's cases probably suffered from what is now recognized as a compensated hemolytic state

Mild icterus may be detected shortly after birth or may be recognized only later in life [120 121] At times the patient is unaware of his abnormality until hyperbilirubinemia is detected on incidental laboratory

defect is probably genetically controlled and may be transmitted as a Mendelian dominant [123, 135]

Finally, this syndrome must be considered in relation to the severe congenital and familial nonhemolytic jaundice (Crigler Najjar syndrome) Although clinically the two conditions differ mainly in the degree of icterus a clear distinction is not always possible Indeed it has been suggested that the two syndromes may represent different degrees of the same defect [120 136] Against this concept is the finding that patients with constitutional hepatic dysfunction form glucuronides at a *normal*

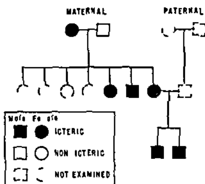



Fig 8-19 Pedigree of a family with constitutional hepatic dysfunction (Gilbert s disease) (*By permission of W G Baroody et al [1*0*]*)

rate and that parents and siblings of patients with congenital nonhemolytic jaundice fail to exhibit mild hyperbilirubinemia This renders it unlikely that constitutional hepatic dysfunction represents the heterozygous form of congenital nonhemolytic jaundice but suggests that the two syndromes are genetically and pathogenetically different

## ICTERUS WITH INCREASED LEVELS OF CONJUGATED BILIRUBIN

### CHRONIC IDIOPATHIC JAUNDICE (DUBIN JOHNSON SYNDROME)

In 1954 Dubin and Johnson [137] and Sprinz and Nelson [138] described a hitherto unrecognized syndrome characterized by chronic icterus and the presence of an unidentified pigment in liver cells Since the original description numerous cases of a similar nature have been observed and reported [139]

The presence of mild and often asymptomatic icterus may be detected in childhood or during adult life [139] Hyperbilirubinemia is usually slight in degree and exhibits marked fluctuation in intensity [138 139] Characteristically the plasma contains a significant fraction of bilirubin which gives a *direct* van den Bergh reaction [138 140] and conjugated

those variants which lie outside the statistical confidence limits of the range of "normal" bilirubin concentrations and thus do not constitute a true abnormality

The finding that the pigment retained in the plasma is always unconjugated bilirubin [80] suggests that the primary defect lies in the conversion of bilirubin to its conjugates As discussed earlier such defects may involve the binding of bilirubin to plasma proteins its transport across the liver cell membrane the structure of the microsomal particles or the activity of the conjugating enzyme system It has been claimed that in some of these patients a reduction in glucuronyl transferase activity can be demonstrated in liver tissue obtained by needle biopsy [131] but details of the technique employed have not been published On the other hand it was found that in vivo such patients are able to form and excrete glucuronides at a rate comparable to that of nonicteric individuals [117 120 132] (Table 8 7) This finding suggests that the glucuronide-forming mechanism can not be significantly impaired and that the hyperbilirubinemia must be due to other defects [117 132]

TABLE 8-7 URINARY EXCRETION OF N ACETYL-*p*-AMINOPHENYL (NAPA) GLUCURONIDE AND TOTAL *p*-AMINOPHENOL IN FIVE MALE PATIENTS WITH CONSTITUTIONAL HEPATIC DYSFUNCTION AFTER A SINGLE INTRAVENOUS INJECTION OF NAPA 10 MG PER KG

| *Patient* | *Serum bilirubin (total mg/100 ml)* | *NAPA glucuronide* | *Total p-aminophenol* |
|---|---|---|---|
| D d I | [illegible] 9 | 27 | 60 |
| T C | 1 5 | 35 | 58 |
| C S | 1 6 | 23 | 62 |
| A D | 2 1 | 46 | 75 |
| J M | 2 8 | 39 | 78 |
| 6 normal males | 0 6 (0 4–0 9) | 32 (25–36) | 61 (4[illegible]–74) |

Amounts excreted in 6 hr in per cent of dose administered

The occurrence of unexplained hyperbilirubinemia in more than one member of a family has repeatedly been reported [120–123 129 130 133–135] Dameshek and Singer [123] described 2 families with 3 and 7 icteric members and among 58 patients with unexplained hyperbilirubinemia studied by Foulk et al [120] 8 had a positive family history for jaundice In the group of 15 patients reported by Alwall et al [135] 55 per cent of the siblings and 26 per cent of the probands parents had bilirubin levels above 1 3 mg per 100 ml The pedigree of a family studied by Baroody et al [129] is reproduced in Fig 8 19 These findings suggest that in some instances of mild chronic hyperbilirubinemia the

defect is probably genetically controlled and may be transmitted as a Mendelian dominant [123 135]

Finally this syndrome must be considered in relation to the severe congenital and familial nonhemolytic jaundice (Crigler Najjar syndrome) Although clinically the two conditions differ mainly in the degree of icterus a clear distinction is not always possible Indeed it has been suggested that the two syndromes may repre ent different degrees of the same defect [120 136] Against this concept is the finding that patients with constitutional hepatic dysfunction form glucuronides at a *normal*

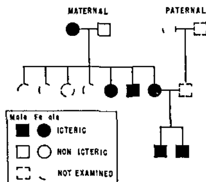



Fig 8-19 Pedigree of a family with constitutional hepatic dysfunction (Gilbert s disease) (*By permission of W G Baroody et al [1 9]*)

rate and that parents and siblings of patients with congenital nonhemolytic jaundice fail to exhibit mild hyperbilirubinemia This renders it unlikely that constitutional hepatic dysfunction represents the hetero zygous form of congenital nonhemolytic jaundice but suggests that the two syndromes are genetically and pathogenetically different

## ICTERUS WITH INCREASED LEVELS OF CONJUGATED BILIRUBIN

### CHRONIC IDIOPATHIC JAUNDICE (DUBIN JOHNSON SYNDROME)

In 1954 Dubin and Johnson [137] and Sprinz and Nelson [138] described a hitherto unrecognized syndrome characterized by chronic icterus and the presence of an unidentified pigment in liver cells Since the original description numerous cases of a similar nature have been observed and reported [139]

The presence of mild and often asymptomatic icterus may be detected in childhood or during adult life [139] Hyperbilirubinemia is usually slight in degree and exhibits marked fluctuation in inten ity [138 139] Characteristically the plasma contains a significant fraction of bilirubin which gives a *direct* van den Bergh reaction [138–140] and conjugated

bilirubin can be demonstrated on paper chromatography [80] In the majority of patients bile pigment is detectable in the urine [139 140]

The hyperbilirubinemia may be entirely asymptomatic or may be associated with weakness increased fatigability and protean gastrointestinal manifestations [139] Enlargement and tenderness of the liver are occasionally observed [139] The results of conventional liver function

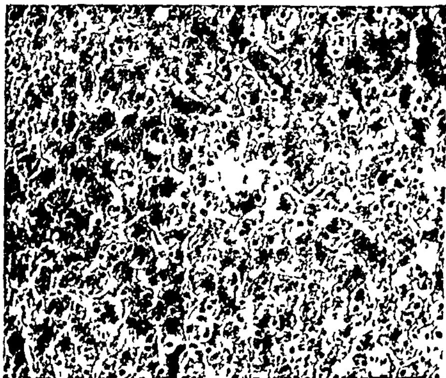


Fig 8-20 Liver in chronic idiopathic jaundice (Dubin-Johnson syndrome) Pigmentation is particularly marked in parenchymal liver cells of the centrolobular area (*By permission of I N Dubin et al* [137])

tests may be either normal or moderately impaired [139 140] In the majority of patients some increase in Bromsulphalein retention is found but it is not known whether this is due to retention in the plasma of unaltered dye or to accumulation of Bromsulphalein metabolites Oral cholecystography frequently fails to display the gallbladder [139] or visualization is only faint [140] Intravenous administration of contrast medium may yield a higher percentage of visualization [139]

Except for the presence of dark pigmentation the gross appearance of the liver is usually normal [139] The tissue core obtained by needle biopsy is often described as green black or dark gray [139 140] On histologic examination of the liver a slight degree of fibrosis or fatty

infiltration may be detectable [139] but the most striking finding is the presence of intracellular coarsely granular brown pigment which is particularly conspicuous in cells of the centrolobular area (Fig 8 20) The pigment tends to be confined to parenchymal cells [138] and to a lesser degree to Kupffer cells [139 140] The nature of this pigment is not known Histochemically it differs from bile pigment hemosiderin hematin and melanin [138 139] It has been suggested that it may belong to the ill defined group of lipofuscins [137, 138 141 142] but in more recent studies this has been disputed [140]

Of importance is the report by Popper s group [140] that in instances of familial occurrence of this syndrome wide variations in the degree of pigmentation may be observed in the liver of different patients In two

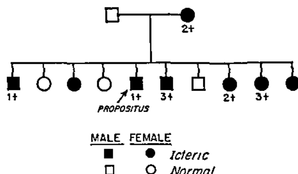



Fig 8-21 Pedigree of a family with chronic idiopathic jaundice The degree of hepatic pigmentation is indicated on an arbitrary scale of 1+ to 3+ In two patients liver biopsies were not performed (*By permission of R L Wolf et al [140]*)

families with 10 icteric members histologic examination of the liver revealed two instances with large amounts of pigment four in which the degree of pigmentation was slight and two with only minimal pigment deposition In the last group the character and quantity of the pigment differed little from the centrolobular pigment often found in parenchymal cells of *normal* livers [143] The e observations are believed to indicate that the accumulation of pigment may not be due to a pecific abnormality but may simply repre ent faulty excretion of a pigment which normally *is formed in the liver and excreted in the bile [140] The postulated* abnormality would include excretion of conjugated bilirubin [139] Brom ulphalein [138–140] rose bengal [140] contrast media employed for cholecystography [139] and the unidentified pigment present in the liver

The syndrome occurs in both sexes and has been observed in widely varying ethnic group including Negroe Persian Jew and Cauca ians [139] A positive family history of icterus is obtained in a ignificant number of patients [139] but a definite familial incidence can be estab-

lished only by systemic performance of liver biopsies on family group [140] The pedigree of a Puerto Rican family studied by Wolf et al [140] is given in Fig 8 21 One parent and seven out of ten siblings exhibited hyperbilirubinemia Liver biopsies performed in all but two of these patients revealed a significant but variable degree of pigmentation This and similar observations [144–146] suggest that the defect may be genetically controlled but additional studies are needed to clarify the exact mode of inheritance

Clinically chronic idiopathic jaundice has many similarities to constitutional hepatic dysfunction Both disorders are characterized by familial incidence chronic and fluctuating hyperbilirubinemia and a benign course In chronic idiopathic jaundice however the presence of an excretory defect and the accumulation of pigment in the liver permit a clear separation from constitutional hepatic dysfunction in which the defect appears to be limited to the conversion of bilirubin to its conjugate In the latter condition the plasma contains only *non*conjugated bilirubin and bilirubinuria is absent whereas in chronic idiopathic jaundice conjugated bilirubin is present in plasma and urine

## OTHER FORMS OF CHRONIC HYPERBILIRUBINEMIA WITH CONJUGATED BILIRUBIN

In 1948 Rotor and his associates [147] described a Philippine family with several icteric members three of whom were studied in detail Mild and fluctuating icterus was discovered shortly after birth or during childhood and persisted throughout life Serum bilirubin concentrations were slightly to moderately increased and the van den Bergh reaction was predominantly direct but bilirubinuria was not noted There was intermittent epigastric discomfort with occasional frank abdominal pain and fever There was nothing to suggest cholelithiasis or increased hemolysis Cholecystography and estimation of erythrocyte life span or fecal urobilinogen excretion were not reported The only significant abnormality was marked retention of Bromsulphalein Liver biopsy performed in one patient showed no significant histologic abnormalities

A pedigree of this family indicates the presence of jaundice in a total of eight members in three generations (Fig 8 22) An additional two members gave a history of jaundice although at the time of examination they were not icteric Unfortunately only three patients were reported in detail [147 148]

Findings similar to those of Rotor et al [147] were reported from Italy where several members of two families were found to have mild chronic elevation of direct reacting serum bilirubin [149–151] Another case probably representing a similar syndrome was recently reported [152] A 29-year old Japanese woman had unexplained asymptomatic icterus with bilirubinuria for at least 11 years Extensive studies revealed the follow

ing the serum bilirubin levels fluctuated from 4 0 to 7 6 mg per 100 ml with approximately half of the pigment present in conjugated form Conventional liver function tests including oral cholecystography failed to reveal abnormal results except for marked retention of Bromsulphalein and a positive cephalin flocculation test Hepatic tissue obtained on two separate occasions showed no histologic abnormalities and pigmentation was absent

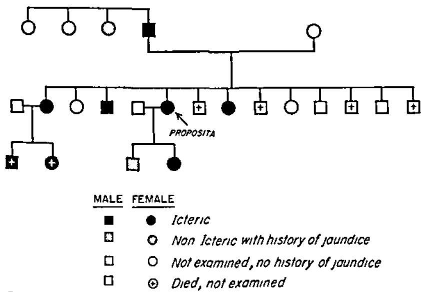



Fig 8-2 Pedigree of a family with chronic hyperbilirubinemia with conjugated bilirubin Liver biopsy was performed in only one patient showing no significant histologic abnormalities (By permission of E Stransky [178])

An older sister of this patient who also has long standing and unexplained icterus had a cholecystectomy 9 years ago Gallstones were present but postoperatively the jaundice persisted without improvement In addition to hyperbilirubinemia the patient also has marked retention of Bromsulphalein

All these patients exhibit findings which suggest a defect in hepatic excretory function The occurrence of icterus in several members of a family may indicate that the disturbance is genetically controlled but the available data are inadequate to establish a definite genetic pattern The absence of pigment in liver cells and the marked abnormality in clearance of Bromsulphalein suggest that the syndrome may differ from chronic idiopathic jaundice but this argument loses force with the recent demonstration that the degree of hepatic pigmentation may be quite variable or indeed minimal in the latter syndrome [140] If more extensive studies confirm these findings it may be difficult or impossible to make a clear

distinction between chronic idiopathic jaundice and the syndrome described in these Philippine Japanese and Italian families A definite classification of the various forms of chronic icterus associated with an hepatic excretory defect must await a better understanding of the fundamental abnormalities involved

## SUMMARY

1 Several syndromes with chronic nonhemolytic icterus have been shown or are suspected to be due to a genetically controlled abnormality in the liver These can be divided into two major groups depending on whether the principal defect involves conversion of bilirubin to its conjugates or excretion of conjugated pigment in the bile The first group comprises patients with congenital familial nonhemolytic jaundice (Crigler Najjar syndrome) and with constitutional hepatic dysfunction (Gilbert s disease) The second group includes chronic idiopathic jaundice (Dubin-Johnson syndrome) and related syndromes (Congenital abnormalities in the development of intrahepatic bile ducts are not discussed here )

2 *Congenital nonhemolytic jaundice* is a rare familial and probably recessively inherited disorder characterized by high concentrations of unconjugated bilirubin in the plasma and reduced pigment excretion in bile and feces Disturbances of the central nervous system resembling kernicterus are frequently present Defects in the glucuronide-forming mechanism have been demonstrated in vivo and in vitro but it is not established with certainty whether the severe hyperbilirubinemia is due solely to reduced function of the conjugating enzyme system or to additional and as yet unknown abnormalities

3 Patients with *constitutional hepatic dysfunction* usually exhibit a much milder degree of hyperbilirubinemia The concentration of unconjugated bilirubin in the serum fluctuates and bilirubinuria is absent Mild and intermittent icterus may be the only manifestation or it may be associated with lassitude increased fatigability and gastrointestinal complaints There is no convincing evidence to suggest that the activity of the glucuronide-forming enzyme system in the liver is reduced and the cause of the hyperbilirubinemia is not yet clear The syndrome frequently occurs in several members of a family suggesting that the metabolic defect may be genetically controlled It is not known whether the same abnormality is present in all cases observed or whether a similar disturbance may be acquired e g in patients who have had infectious hepatitis

4 *Chronic idiopathic jaundice* is a syndrome of possible hereditary nature, primarily involving hepatic excretory function The plasma contains increased concentration of conjugated bilirubin Bilirubinuria is frequently observed and the liver often exhibits intracellular deposition

of an unidentified pigment A few instances of a similar syndrome but lacking pigmentation of the liver have been described in families of Philippine Italian and Japanese origin The relationship of these cases to chronic idiopathic jaundice is not clear

## BIBLIOGRAPHY


1 Adams F *The Genuine Works of Hippocrates* Williams & Wilkins Baltimore 1939
2 Gray C H *The Bile Pigments* Methuen London 1953
3 Virchow R Die pathologischen Pigmente Arch path Anat 1 379 1847
4 Rich A R The formation of bile pigment Physiol Rev 5 182 1925
5 Aschoff L Das reticulo-endotheliale System und seine Beziehungen zur Gallenfarbstoffbildung Munchen med Wchnschr 69 1352 1922
6 McNee J W Jaundice a review of recent work Quart J Med 16 390 1923
7 Whipple G H and Hooper C W Bile pigment output influenced by hemoglobin injections anemia and blood regeneration Am J Physiol 43 258 1917
8 Mann F C Sheard C Bollman J L and Blades E J The formation of bile pigment from hemoglobin Am J Physiol 76 306 1926
9 Tarchanoff J F Ueber die Bildung von Gallenpigment aus Blutfarbstoff im Thierkoerper Arch ges Physiol 9 53 1874
10 Fischer H and Orth H *Die Chemie des Pyrrols* Akademische Verlagsgesellschaft m b H Leipzig 1937
11 Minkowski O and Naunyn B Beitraege zur Pathologie der Leber und des Icterus 2 Ueber den Icterus durch Polycholie und die Vorgaenge in der Leber bei demselben Arch exper Path u Pharmacol 21 1 1886
12 Hijmans van den Bergh A A and Mueller P Ueber eine direkte und indirekte Diazoreaktion auf Bilirubin Biochem Ztschr 77 90 1916
13 Watson C J The pyrrol pigments with particular reference to normal and pathological hemoglobin metabolism in *Handbook of Hematology* edited by Hal Downey Hamilton London 1938
14 With T K *Biology of Bile Pigments* Arne Forst Hansen Copenhagen 1954
15 Schmid R Direct reacting bilirubin bilirubin glucuronide in serum bile and urine Science 124 76 1956
16 Billing B H Cole P G and Lathe G H The excretion of bilirubin as a diglucuronide giving the direct van den Bergh reaction Biochem J 65 774 1957
17 Talafant E Properties and composition of the bile pigment giving a direct diazo reaction Nature London 2 312 1956
18 Overbeek J T G Vink C L J and Deenstra H The solubility of bilirubin Rec trav chim 74 81 1955
19 Wintrobe M M *Clinical Hematology* Lea & Febiger Philadelphia 1956
20 Jandl J H Jones A R and Castle W B The destruction of red cells by antibodies in man I Observations on the sequestration and lysis of red cells altered by immune mechanisms J Clin Invest 36 14 8 1957
21 Lepehne G Das Problem der Gallenfarbstoffbildung inne halb und usserhalb der Leber Folia haemat 39 277 1930
22 Moore C V and Dubach R Metabolism and requirements of iron in man J A M A 162 197 1956
23 Hawkins W B and Johnson A C Bile pigment and hemoglobin interrelation in anemic dogs Am J Physiol 126 3 6 1939
24 Schmid R Some aspects of bile pigment metabolism Clin Chem 3 Suppl 394 1957
25 Nencki M and Zaleski J Untersuchungen ueber den Blutfarbstoff Zt chr physiol Chem 30 384 1900
26 Eppinger H *Die Hepatolienal n Erkrankungen* Springer Berlin 1920

27 Bingold K Haemolyse Blutfarbstoffabbau Haematinaemie und Ikterus Arch klin Med 97 257 1921

28 Schumm O Haematin als pathologischer Bestandteil des Blutes Ztschr physiol Chem 97 32 1916

29 Fairley N H Methaemalbumin clinical aspects Quart J Med 10 95 1941

30 Brugsch J and Retzlaff K Blutzerfall Galle und Urobilin Ztschr exper Path 11 508 1912

31 Liss I J Schwartz S and Watson C J The conversion of hematin to bilirubin following intravenous administration in human subjects J Clin Invest 24 283 1945

32 London I M The conversion of hematin to bile pigment J Biol Chem 184 373 1950

33 Kench J E Gardikas C and Wilkinson J F Bile pigment formation in vitro from haematin and other haem derivatives Biochem J 47 1 3 1950

34 Libowitzky H Ueber Koproesterverdohaemin und seine Umwandlung in Koprobilin Ztschr physiol Chem 265 191 1940

35 Fischer H and Libowitzky H Ueberfuehrung von Kopro-haemin I in Koproglaukobilin Ztschr physiol Chem 251 198 1938

36 Lemberg R and Legge J W *Haematin Compounds and Bile Pigments* Interscience New York 1949

37 London I M Yamasaki M and Sibella A G Conversion of Protoporphyrin to Bile Pigment Fed Proc 10 217 1951

38 Heikel T Lockwood W H and Rimington C Formation of non-enzymatic haem Nature London 182 313 1958

39 *Mauzerall D and Granick S Porphyrin biosynthesis in erythrocytes III* Uroporphyrinogen and its decarboxylase J Biol Chem 232 1141 1958

40 Fischer H and Herrle K Einwirkung von Licht auf Porphyrine Ueberfuehrung von Aetioporphyrin I in bilirubinoide Farbstoffe Ztschr physiol Chem 251 85 1938

41 Mills G C and Randall H P Hemoglobin catabolism II The protection of hemoglobin from oxidative breakdown in the intact erythrocyte J Biol Chem 232 589 1958

42 Kaziro K Kikuchi G and Hanaoka C Verdohemoglobin an immediate precursor of biliverdin in the model reaction of hemoglobin in vitro J Biochem 42 423 1955

43 Lemberg R and Legge J W Intracorpuscular bile pigment formation Australian J Exper Biol & M Sc 20 65 1942

44 Gajdos A and Tiprez G La signification biologique des corpuscules de Heinz Compt rend soc biol 139 545 1945

45 Kiese M and Seipelt L Bildung und Elimination von Verdoglobin Arch exper Path 200 648 1943

46 Barkan G and Walker B S Red blood cell as source of iron and bilirubin of the blood plasma J Biol Chem 131 447 1939

47 Lemberg R Bile pigments from normal erythrocytes Nature London 163 97 1949

48 Gardikas C Kench J E and Wilkinson J F Choleglobin formation in the erythrocyte Nature London 161 607 1948

49 Gardikas C Kench, J E and Wilkinson J F Bile pigment precursors in normal human erythrocytes Biochem J 46 85 1950

50 Ottenberg R Bilirubin and bile salts in jaundice J Mt Sinai Hosp 9 937 1943

51 With T K The bilirubin production of the human organism and its significance to the pathogenesis of jaundice Acta med Scandinav 123 166 1946

52 Deenstra H L excrétion des pigments biliaires par les reins Ann méd 51 685 1950

53 Crigler J F and Najjar V A Congenital familial nonhemolytic jaundice with kernicterus Pediatrics 10 169 1952

54 Schmid R *Congenital Defects in Bilirubin Metabolism in Liver Function* publication 4 American Institute of Biological Sciences Washington 1958

55 Axelrod J Schmid R and Hammaker L A biochemical lesion in congenital non-obstructive non hemolytic jaundice Nature London 180 14?? 1957
56 Siedel W v Poelnitz W and Eisenreich F Bilifuscin und Mesobilifuscin als natuerliche Abbauprodukte des Blutfarbstoffes ueber Vorkommen und Bildung Naturwissenschaften 34 314 1947
57 Siedel W Stich W and Eisenreich F Promesobilifuscin (Mesobilileukan) ein neues physiologisches Abbauprodukt des Blutfarbstoffes Naturwissenschaften 35 316 1948
58 Gilbertsen A S Lowry P T Hawkinson V and Watson C J Studies of the dipyrrylmethene ( fuscin ) pigments 1 The anabolic significance of the fecal mesobilifuscin J Clin Invest 38 1166 1959
59 Bingold K Eigenschaften und physiologische Bedeutung des Pentdyopents Klin Wchnschr 17 289 1938
60 Hulst L A and Grotepass W Uber das Pentdyopent von Bingold Klin Wchnschr 15 201 1936
61 Schmid R Brecher G and Clemens T Familial hemolytic anemia with erythrocyte inclusion bodies and a defect in pigment metabolism Blood 14 991 1959
62 With T K Occurrence in human serum of yellow substances different from bilirubin Nature London 158 310 1946
63 Lups S and Meyer F G D Some investigations about the occurrence of pigments that give no diazo reaction in jaundiced sera Acta med scandinav 126 85 1946
64 Bingold K Blutkatalase und Wasserstoffsuperoxyd als wirkende Kraefte beim Blutfarbstoffabbau Ergebn inn Med u Kinderheilk 60 1 1941
65 Haurowitz F Schwerin P and Yensen M M Destruction of hemin and hemoglobin by the action of unsaturated fatty acids and oxygen J Biol Chem 140 353 1941
66 Cruz W O Hawkins W B and Whipple G H Acetylphenylhydrazine anemia 2 Bile pigment elimination and new hemoglobin reconstruction in the bile fistula dog Am J M Sc 203 848 1942
67 London I M West R Shemin D and Rittenberg D On the origin of bile pigment in normal man J Biol Chem 184 351 1950
68 Gray C H Neuberger A and Sneath P H A Incorporation of $N^{15}$ in the stercobilin in the normal and the porphyric Biochem J 47 87 1950
69 Theorell H Béznak M Bonnichsen R Paul K G and Akeson A Distribution of injected radioactive iron in guinea pigs and its rate of appearance in hemoproteins and ferritins Acta chem scandinav 5 445 1951
70 Gray C H and Scott J J The effect of haemorrhage on the incorporation of glycine 2-$C^{14}$ into stercobilin Biochem J 71 38 1959
71 London I M West R Shemin D and Rittenberg D Porphyrin formation and hemoglobin metabolism in congenital porphyria J Biol Chem 184 365 1950
72 London I M and West R The formation of bile pigment in pernicious anemia J Biol Chem 184 359 1950
73 Baumgartel T Zur fermentativen Reduktion der Gallenfarbstoffe Bilirubin und Biliverdin Klin Wchnschr 27 77 1949
74 Martin N H Preparation and properties of plasma proteins 21 Interactions with bilirubin J Am Chem Soc 71 1230 1949
75 Cohn E J The chemical specificity of the interaction of diverse human plasma proteins Blood 3 471 1948
76 Zieve L Hill E Hanson M C L Falcone A B and Watson C J Normal and abnormal variations and clinical significance of the one minute and total serum bilirubin determinations J Lab Clin Med 38 446 1951
77 Isselbacher K J and McCarthy E A Studies on bilirubin sulfate and other nonglucuronide conjugates of bilirubin J Clin Invest 38 645 1959
78 Schmid R Axelrod J Hammaker L and Swarm R L Congenital jaundice in rats due to a defect in glucuronide formation J Clin Invest 37 1123 1958

79 Brown A K and Zuelzer W W Studies on the neonatal development of the glucuronide conjugating system J Clin Invest 37 332 1958
80 Schmid R Unpublished observation
81 Billing B H and Lathe G H Bilirubin metabolism in jaundice Am J Med 24 111 1958
82 *Schmid R Jaundice and bilirubin metabolism A M A Arch Int Med* 101 669 1958
83 Schachter D Nature of the glucuronide in direct reacting bilirubin Science 126 507 1957
84 Schmid R Hammaker L and Axelrod J The enzymatic formation of bilirubin glucuronide Arch Biochem 70 285 1957
85 Dutton G J and Storey I D E Uridine compounds in glucuronic acid metabolism I The formation of glucuronides in liver suspensions Biochem J 57 275 1954
86 Smith E E B and Mills G T Uridine nucleotide compounds of liver Biochim biophys acta 13 386 1954
87 Strominger J L Kalckar H M Axelrod J and Maxwell E S Enzymatic oxidation of uridine diphosphate glucose to uridine diphosphate glucuronic acid J Am Chem Soc 76 6411 1954
88 Axelrod J Inscoe J K and Tomkins G M Enzymatic synthesis of N glucosyluronic acid conjugates J Biol Chem 232 835 1958
89 Neufield E F Feingold D S and Hassid W Z Enzymatic phosphorylation of D-glucuronic acid by extracts from seedlings of *Phaseolus aureus* Arch Biochem 83 96 1959
90 *Lathe G H and Walker M The synthesis of bilirubin glucuronide in animal and human liver* Biochem J 70 705 1958
91 Grodsky G M and Carbone J V Synthesis of bilirubin glucuronide by tissue homogenates J Biol Chem 226 449 1957
92 Dutton G J Glucuronide synthesis in foetal liver and other tissue Biochem J 71 141 1959
93 Bollman J L in Panel Bilirubin metabolism Gastroenterology 36 1313 1959
94 Weinbren K and Billing B H Hepatic clearance of bilirubin as an index of cellular function in the regenerating rat liver Brit J Exper Path 37 199 1956
95 Schachter D Estimation of bilirubin mono and diglucuronide in the plasma and urine of patients with nonhemolytic jaundice J Lab Clin Med 53 557 1959
96 Lathe G H Claireaux A E and Norman A *Recent Advances in Paediatric* No 4 Little Brown Boston 1958
97 Karunairatnam M C Kerr L M H and Levvy G A Glucuronide-synthesizing system in mouse and its relationship to β glucuronidase Biochem J 45 496 1949
98 *Hartiala K J V and Pulkkinen M Studies on detoxication mechanisms IV* Glucuronide synthesis in the fetal rabbit Ann med exper et biol Fenniae 33 246 1955
99 Grodsky G M Carbone J V and Fanska R Enzymatic defect in metabolism of bilirubin in fetal and newborn rat Proc Soc Exper Biol & Med 97 291 1958
100 Schmid R Buckingham S Mendilla G A and Hammaker L Bilirubin metabolism in the foetus Nature London 183 1823 1959
101 Fouts J R and Adamson R H Drug metabolism in the newborn rabbit Science 129 897 1959
102 Gunn C K *Hereditary acholuric jaundice J Her* 29 137 1938
103 Malloy H T and Loewenstein L Hereditary jaundice in the rat Canad M A J 42 122 1940
104 Blanc W A and Johnson L Studies on kernicterus J Neuropath & Exper Neurol 18 165 1959
105 Carbone J V and Grodsky G M Constitutional nonhemolytic hyper

bilirubinemia in the rat defect of bilirubin conjugation Proc Soc Exper Biol & Med 94 461 1957
106 Childs B and Najjar V A Familial non hemolytic jaundice with kernicterus Report of two cases without neurologic disease Pediatrics 18 369 1956
107 Rosenthal I M Zimmerman H J and Hardy N Congenital nonhemolytic jaundice with disease of central nervous system Pediatrics 18 378 1956
108 Jervis G A Constitutional nonhemolytic hyperbilirubinemia with findings resembling kernicterus A M A Arch Neurol & Psychiat 81 55 1959
109 Klingberg W G Washington University St Louis Mo Personal communication
110 Enderlen E Thannhauser S J and Jenke M Die Einwirkung der Leber exstirpation bei Hunden auf den Cholesterinstoffwechsel Beobachtung ueber ein gelbes Pigment im Blute nach Leberexstirpation (Xanthorubin) Arch exper Path u Pharmakol 120 16 1927
111 Josephs H W Urobilin excretion in infancy and childhood Relation to blood destruction and formation Bull Johns Hopkins Hosp 55 154 1934
112 Peterson R E and Schmid R A clinical syndrome associated with a defect in steroid glucuronide formation J Clin Endocrinol 17 1485 1957
113 Childs B Sidbury J B and Migeon C J Glucuronic acid conjugation by patients with familial non hemolytic jaundice and their relatives Pediatrics 23 903 1959
114 Isselbacher K J and Axelrod J Enzymatic formation of corticosteroid glucuronide J Am Chem Soc 77 1070 1955
115 Schachter D and Manis J G Salicylate and salicyl conjugates fluorimetric estimation biosynthesis and renal excretion in man J Clin Invest 37 800 1958
116 Arias I M and London I M Bilirubin glucuronide formation in vitro demonstration of a defect in Gilbert s disease Science 126 563 1957
117 Schmid R and Hammaker L Glucuronide formation in patients with constitutional hepatic dysfunction (Gilbert s disease) New England J Med 260 1310 1959
118 Schmid R The identification of direct reacting bilirubin as bilirubin glucuronide J Biol Chem 229 881 1957
119 Gilbert A Lereboullet P and Herscher M Les trois cholémies congénitales Bull et mém Soc méd hop Paris 24 1203 1907
120 Foulk W T Butt H R Owen C A Whitcomb F F and Mason H L Constitutional hepatic dysfunction (Gilbert s disease) its natural history and related syndromes Medicine 38 25 1959
121 Meulengracht E Icterus intermittens juvenilis (chronischer intermittierender juveniler Subicterus) Klin Wchnschr 18 118 1939
122 Alwall N On hereditary non hemolytic bilirubinemia Acta med scandinav 123 560 1946
123 Dameshek W and Singer K Familial non hemolytic jaundice constitutional hepatic dysfunction with indirect van den Bergh reaction Arch Int Med 67 259 1941
124 Comfort M W Constitutional hepatic dysfunction Proc Staff Meet Mayo Clin 10 57 1935
125 Rosendaal H M Comfort M W and Snell A M Slight and latent jaundice significance of elevated concentrations of bilirubin giving indirect van den Bergh reaction J A M A 104 374 1935
126 Hult H Cholémie simple familiale (Gilbert) and posthepatitic state without fibrosis of liver Acta med scandinav 138 Suppl 244 1 1950
127 Kalk H and Wildhirt E Die posthepatitische Hyperbilirubinaemie (der sog erworbene haemolytische Ikterus nach Hepatitis) Ztschr klin Med 153 354 1955
128 Volwiler W and Elliott J A Jr Late manifestations of epidemic infectious hepatitis Gastroenterology 10 349 1948
129 Barondy W C and Shugart R T Familial non hemolytic icterus Am J Med 20 314 1956

130 Carithers H A Jr Non hemolytic familial jaundice J Pediatrics 19 81 1941
131 Arias I M Gilbert s disease Bull New York Acad Med 35 450 1959
132 Barniville H T F and Mi k R Urinary glucuronic acid excretion in liver disease and the effect of a salicylamide load Brit M J 1 337 1953
133 Manson J S Hereditary icterus or familial acholuric jaundice Brit M J 1 131 1928
134 Tecon R M A propos d ictères chroniques Helvet med acta 5 671 1938
135 Alwall N Laurell C B and Nilsby I Studies on heredity in cases of non hemolytic bilirubinemia without direct van der Bergh reaction (hereditary non hemolytic Bilirubinemia) Acta med scandinav 124 114 1946
136 Arias I M in Land Bilirubin metabolism Gastroenterology 36 1318 1959
137 Dubin I N and Johnson F B Chronic idiopathic jaundice with unidentified pigment in liver cells new clinico pathologic entity with report of 1 cases Medicine 33 155 1954
138 Sprinz H and Nelson R S Persistent nonhemolytic hyperbilirubinemia associated with lipochrome-like pigment in liver cells report of 4 cases Ann Int Med 41 952 1954
139 Dubin I N Chronic idiopathic jaundice A review of 50 cases Am J Med 24 268 1958
140 Wolf R L Pizette M Richman A Dreiling D A Jacobs W Fernandez O and Popper H Chronic idiopathic jaundice A study of two afflicted families Am J Med 28 32 1960
141 Brown N L and Shnitka T K Constitutional non hemolytic jaundice with lipochrome hepatosis (Dubin-Sprinz disease) Am J Med 21 292 1956
142 Hamperl H Chronischer nichthaemolytischer Ikterus mit Ablagerung eines eigentuemlichen Pigmentes in der Leber Klin Wchnschr 35 177 1957
143 Post J Benton J G and Breakstone R Observations on a cytoplasmic hepatic cell pigment in man A M A Arch Path 52 6J 1951
144 Beker S and Read A E Familial Dubin-Johnson syndrome Gastroenterology 35 387 1958
145 John G G and Knudtson K P Chronic idiopathic jaundice two cases occurring in siblings with histochemical studies Am J Med 21 138 1956
146 Warmoes F DeDeurwaerder R J and Van den Eynde I Un cas d ictère de Dubin-Johnson Acta gastro-enterol belg 20 5 5 1957
147 Rotor A B Manahan L and Florentin A Familial non hemolytic jaundice with direct van den Bergh reaction Acta med philippina 5 37 1948
148 Stransky E Uber kongenitalen familiaeren nichthaemolytischen Ikterus Ann paediat 175 301 1950
149 Dagnini G and Morechi E Ittero familiare epatogeno Recenti progr med 23 47 1957
150 Canali G Ittero congenito familiare epatico Recenti progr med 23 69 1957
151 Cinotti G A Fabiani F and Pericoli F Ittero anemolitico familiare a bilirubinemia diretta e splenomegalia Policlinico (sez prat) 64 1573 1957
152 Schiff L Billing B H and Oikawa Y Familial nonhemolytic jaundice with conjugated bilirubin in the serum New England J Med 260 1315 1959

Part Three

# Diseases Primarily Related to Disorders of Amino Acid Metabolism

---

# Chapter 9

# Familial Goiter*

---

*John B Stanbury*

This chapter is concerned with the metabolic aspects of familial goiter. The patients to be discussed have, with few exceptions, retarded mental or skeletal development and, if untreated with thyroid substance, show the outward appearance of thyroid hormone deficiency. Goiter may be present at birth but more commonly becomes noticeable during childhood. Siblings may be similarly afflicted. These patients are nosologically distinguished from clinically similar patients who have endemic goiter. Several reviews have appeared [*1–6*] since the first cases were reported by Osler in 1897 [7].

The results of laboratory investigations of many subjects with familial goiter and hypothyroidism have made it possible to classify some of them into categories according to specific and identifiable biochemical lesions. In each category the lesion is specific and distinct, but the net effect is the same: synthesis and delivery of thyroid hormone are inadequate. Before considering these groups in turn, it is appropriate to survey briefly the principal metabolic pathways of iodine in the body. These pathways are illustrated in Fig. 9.1. Competent reviews are available elsewhere [*8–12*].

## METABOLISM OF IODINE

### ABSORPTION AND DISTRIBUTION

Iodide[1] is absorbed through the gastrointestinal tract as inorganic iodide and is rapidly distributed throughout the extracellular fluid of the body. Free iodine and the iodine of most organic compounds is reduced

* This work was supported in part by grant from the Public Health Service (A 1880).

[1] In this chapter the term *iodine* is used in a generic sense to encompass all forms and oxidation states unless otherwise indicated. Similarly, the term *labeled iodine* is used generically to indicate the iodine which is marked by a radioactive isotope of iodine. The term *iodide* always indicates the reduced ionic form of the element.

to iodide during digestion and absorption The volume of distribution is approximately 28 per cent of body weight at 1 hr Iodide penetrates the red cells to approximately 65 per cent of plasma concentration and also enters the bones to a detectable degree Iodinated thyronines and tyrosines may be absorbed intact but the latter are largely deiodinated prior to absorption Early after absorption thyroxine ($T_4$) and 3 5 3 triiodothyronine ($T_3$) are confined to the vascular compartment because of *binding to carrier proteins in the plasma Certain iodinated dyes such* as those used in the x ray visualization of the biliary tree are also absorbed unchanged

Fig 9-1 Schema of the metabolism of iodine in normal man The solid arrows represent the direction of metabolism Vertical arrows pointing upward indicate stimulation of a step and *those pointing downward indicate inhibition* TriAc and TetrAc are the acetic acid analogues of tri and tetraiodothyronine respectively

Except in the postabsorptive state the iodide concentration of the serum is usually less than 1 μg per 100 ml The inorganic iodide of the *plasma is removed almost* entirely by the kidney and the thyroid The relative rates of clearance by these two organs determine the value of the familiar radioactive iodine ($I^{131}$) uptake test for thyroid function Renal clearance of iodide is normally of the order of 35 ml per min and appears to be independent of iodine supply in man but not in dog or rat and is largely independent of chloride loading [13] Thyroidal clearance of iodide varies widely depending upon the functional state of the gland but

normally lies between 10 and 35 ml per min The small quantities of iodide removed from the plasma by the salivary and gastric glands are returned to the plasma after absorption in the small bowel Small amounts of iodide are removed by the mammary glands during lactation Losses of inorganic iodide in feces sweat and expired air are small

### IODIDE TRAPPING

Iodide cleared from the plasma by the thyroid gland is retained momentarily in the gland as diffusible and dialyzable inorganic iodide Only 1 to 2 per cent of the iodine in the gland fails to precipitate upon treatment with trichloracetic acid This small amount is in much higher concentration than is the iodide of the plasma In spite of the concentration gradient the inorganic iodide of the gland is in free and ready exchange with the plasma iodide Thus if the inorganic iodide of the gland is labeled with $I^{131}$ the latter can be diluted out of the gland almost instantaneously by administration of a large amount of unlabeled iodide

Iodide trapping is oxygen dependent and inhibited by cyanide azide and a number of other substances which interfere with oxidative processes in cell as well as by sulfhydryl inhibitors [*14 15*] The mechanism of transport across the cell wall against a concentration gradient is entirely unknown Trapping requires an expenditure of energy and is doubtless enzymatically controlled as are probably most other biochemical processes in the gland Oxidative phosphorylation is involved in the trapping of iodide [*15*]

The normal resting thyroid maintains a concentration of iodide against the plasma of approximately 20 1 This may be increased to several hundred to one in the hyperplastic gland The magnitude of the thyroid/plasma ratio is determined by the thyrotropic hormone but the ratio is also governed by the quantity of stored iodine within the gland itself [*16 17*] The ratio is constant over wide plasma concentration ranges of iodide but with increasing concentration the ratio eventually falls toward unity as the holding capacity of the gland approaches saturation

Iodide trapping has been studied in model systems on the presumption that the iodide is held in simple anionic electrostatic binding to intracellular (or cell surface) proteins Homogenate of thyroid tissue within a semipermeable bag concentrated iodine from the surrounding bath [*18 19*] but there was no excess in the bag as inorganic iodide The accumulated iodine was found in part as monoiodotyrosine (MIT) and in part as an unidentified component which was extractable slowly into carbon disulfide and accordingly was also in an oxidized form Accumulation was blocked by reducing agents and by heat inactivation but not by perchlorate Inhibition by catalase indicated that a peroxidase is involved in the process Thus the model system failed to imitate the in vivo iodide trap but suggested the existence of an intermediate bound form of oxidized

iodine This might conceivably be a storage form either firmly adsorbed or in molecular linkage

Thiocyanate ($SCN^-$) and perchlorate ($ClO_4^-$) ions prevent iodide trapping [20, 21] perchlorate is about ten times as potent as thiocyanate ✓ If either of these substances is administered after trapping of iodide has occurred trapped iodide is rapidly discharged from the gland but iodine which has been chemically bound to iodinated tyrosines or thyronines is retained Chronic administration of $SCN^-$ may lead to the development of goiter as was observed in certain patients treated with this substance for hypertension It is not known whether $SCN^-$ and $ClO_4^-$ block trapping by interfering with cell membrane transport or by competing for binding sites within the gland Preferential concentration of these ions by the thyroid has not been demonstrated with certainty [22 23]

OXIDATION AND ORGANIFICATION OF IODIDE

The next step after iodide trapping in hormone synthesis is the oxidation of iodide and its displacement of a hydrogen from the 3 position of tyrosyl residues The tyrosine is thought to be in peptide linkage because organically bound iodine is precipitated with protein precipitants such as trichloracetic acid The degree of complexity of this step is undetermined There may simply be an oxidation of iodide with iodination of tyrosyl residues proceeding *pari passu* or there may be an intermediate storage form of iodine which requires a transferring enzyme in order to gain access to tyrosine Free or peptide-linked tyrosine is iodinated by iodine nonenzymatically *in vitro* but it is entirely possible that the process is rate-governed enzymatically in the gland

The nature of iodide oxidation in the thyroid has been a particularly baffling problem Iodide is oxidized both by slices *in vitro* and by cell free homogenates [19 24–26] and this oxidation is abolished by heat Inhibition of iodide oxidation in thyroid slices by cyanide, azide sulfide and carbon monoxide was interpreted by Schachner et al [26] as indicating that cytochrome oxidase is involved but they could not eliminate the possibility of participation by a peroxidase ✓

There seems to be good evidence that peroxidase is concerned in iodide oxidation ✓ Not only has peroxidase activity been demonstrated many times in thyroid tissue [27–29] but also the oxidation of iodide which proceeds rather specifically in thyroid tissue is inhibited by catalase [19 30] Further a variety of substances which inhibit organification of iodide in vivo or by thyroid slices such as thiourea and resorcinol either inhibit the peroxide-peroxidase system or act as competitive substrates for it [31 32] Finally there is a small but nonetheless necessary oxygen requirement [26] There seems little merit in the counterargument that a peroxidase has not been extracted from the thyroid because the requirement for oxidizing the minute amounts of iodide needed is very small

indeed The inhibition by catalase indicates at least that peroxide is formed in the system For a discussion of in vivo generation of peroxide see Chap 46

It has been suggested that the only requirements for iodide oxidation are large amounts of iodide and oxidizing conditions [11] and by implication that there may be no specific iodide oxidizing system in the thyroid gland The colloid has a high oxidation reduction potential and this is lowered by substances which have antithyroid activity such as thiouracil [27] The possibility remains that a specific electron transfer system exists for converting iodide to iodine Recently this possibility has been reopened by Alexander [33] who has described a specific iodide peroxidase which catalyzes the coupled peroxidation of iodide and is present in thyroid and salivary gland homogenates The enzyme is a tissue component rather than a contaminant from the hemoproteins of the blood Its action is inhibited by cyanide and thiourea but not by perchlorate

The tyrosine iodinase system of Fawcett and Kirkwood [34] requires added tyrosine and cupric ion as the oxidant Since the organification of iodide fails to take place when cupric ion is added to boiled tissue [19] one cannot consider this ion as the normal specific oxidant of iodide More recently Serif and Kirkwood [30] have described a second system containing particulates from thyroid homogenates which oxidized iodide This system is identical with or very similar in its properties to their earlier soluble system The requirement for cupric ion is at variance with results obtained by others [19 24 35] although all have reported enhancement of oxidation and binding with copper

Whatever the mechanism of iodide oxidation and transfer to tyrosyl residues may be homogenates of thyroid tissue readily produce MIT and probably small amounts of diiodotyrosine (DIT) as well [34–36] In addition two iodinated compounds of unknown structure and significance appear [37] Formation of all these compounds is much enhanced by addition of flavine nucleotides to the system [38] but an effect of pyridine nucleotides is not established [35 36] DPN appears to stimulate the production of still another unidentified iodinated component [38] The MIT formed in homogenate systems is in peptide linkage and is released as the free amino acid by proteolytic hydrolysis The poor yield of DIT has suggested that a different iodinating system is required for the iodination of MIT than for MIT formation [39]

## COUPLING REACTION

The iodothyronines $T_3$ and $T_4$ are probably formed by coupling of two molecules of iodotyrosine and extrusion of a side chain [40] There is little to support the alternative hypothesis that the iodothyronines are formed by iodination of thyronine This amino acid has never been found in thyroid tissue [11] Coupling is thought to occur while the respective

amino acids are in peptide linkage rather than in free form The condensation proceeds slowly in vitro when only DIT is present in slightly alkaline solution [41] and is accelerated when a source of oxygen is present or when the carboxyl or amino group or both is covered in peptide linkage [42 43] The reaction may be catalyzed by iodine [42] Interestingly, coupling of N acetyldiiodotyrosine is inhibited by thiourea [44]

The reaction may depend upon the production of free iodine within the gland as described above or a more specific mechanism may be involved It has been proposed that condensation first involves oxidative formation of a quinoid intermediate which becomes a free radical which then unites with a second iodotyrosine molecule to form an iodothyronine and to split off pyruvic acid and ammonia [43]

No enzyme which promotes iodotyrosine coupling has been described Failure of coupling to proceed in cell free homogenate systems whereas $T_4$ forms fairly readily in slices incubated with $I^{131}$ strongly suggests that $T_4$ formation in vivo either is enzymatically controlled or requires a high degree of cellular organization Addition of a wide variety of nucleotide and other cofactors to homogenates has failed to promote the formation of $T_3$ or $T_4$ [24 35 36 45]

Both $T_3$ and $T_4$ are formed in the thyroid gland $T_3$ could be formed either by deiodination of $T_4$ or by the coupling of one molecule of MIT with one of DIT [12] Roche and his colleagues favor coupling of one molecule of DIT and MIT since there was no prior incorporation of $I^{131}$ into either $T_3$ or $T_4$ and since no $T_4$ deiodinase has been detected in thyroid tissue Roche et al have also reported two other products of coupling in the thyroid i e 3 3 diiodothyronine and 3 3,5 triiodothyronine [46 47] The presence of these two compounds in the thyroid or blood has not been confirmed and there is indirect evidence against their appearance in the blood at least in significant amounts [48 49]

## HORMONE STORAGE AND RELEASE

Thyroxine ($T_4$) and 3 5 3 triiodothyronine ($T_3$) are retained in the thyroid gland in storage form within the colloid as peptide-linked residues within the specific thyroid protein thyroglobulin Thyroglobulin is a glycoprotein which has a molecular weight of approximately 650 000 and an isoelectric point of 4 6 and which salts out of phosphate buffer between 1 4 and 1 75 M concentration

Thyroglobulin is the principal iodinated component of the thyroid but at least two other iodinated components are present in much smaller concentration [50 51] One of these is associated with the nuclear fraction of homogenate of thyroid tissue the other is soluble but has a slower sedimentation constant than thyroglobulin Their role in thyroid function is not yet apparent Ingbar et al [52] have evidence from ion exchange

chromatography that preparations of sheep thyroglobulin made by salting-out techniques are not homogeneous. The same is true of human thyroglobulin when subjected to electrophoresis on hydrolyzed starch, but the labeled iodine is almost entirely confined to a single sharp band which is the major protein component [53].

One of the principal unsolved problems of the thyroid gland is the nature of thyroglobulin with respect to the iodinated amino acids. There is every reason to suspect that thyroglobulin may contain widely varying amounts of these amino acids, and yet such variability in amino acid content and sequence is not in keeping with current concepts of the specificity of protein structure. It may be that amino acid sequence is fixed and that degree of iodination can vary, or that the iodinated amino acids are at N terminal or C terminal positions and can vary without altering the backbone of the thyroglobulin molecule. Alternatively, it may be that thyroglobulin is generated as a highly specific structure closely dependent upon its template and that heterogeneity is introduced by breaking and remaking peptide chains in the course of the coupling reaction. Roche et al. [54] found that some of the $T_4$, DIT, and tyrosine occupy N terminal positions of pork thyroglobulin. Most of the DIT and $T_4$ were internal. Glutamic acid, serine, lysine, alanine, leucine, and small amounts of threonine and valine were also found at N terminal positions. $T_3$ and MIT were not found at N terminal positions. Pancreatin releases MIT first, followed by DIT from thyroglobulin; $T_3$ and $T_4$ came off more slowly [55]. Little else is known of the fine structure of thyroglobulin.

The strongly stimulated thyroid gland may possibly secrete newly formed thyroid hormones directly into the blood without going through a thyroglobulin stage [56], but under normal circumstances $T_4$ and $T_3$ are released as needed from the thyroglobulin. Degradation of thyroglobulin is brought about through a sequence of lytic enzymes [57–60] which in turn may be activated by the thyrotropic hormone [61]. The protease and peptidase which have been extracted from the thyroid lack specificity for thyroglobulin. The protease has a pH optimum of 3.5 and is much more effective against hemoglobin than thyroglobulin. The peptidase has a pH optimum near neutrality.

Proteolysis releases $T_4$ and $T_3$ for diffusion into the blood. MIT and DIT, also present in the thyroglobulin, are also released, but are deiodinated by a potent deiodinase which is present in the thyroid parenchymal cells [62–64]. This deiodinase is a microsomal enzyme which requires TPNH as a cofactor [65, 66]. The removal of the first iodine from DIT occurs less rapidly than does the deiodination of MIT. The end products of the reaction are iodide and tyrosine. The freed iodide presumably is conserved for reutilization by the parenchymal cells. Iodotyrosine deiodinase has also been demonstrated in liver, kidney, and other tissue. The presence of this enzyme in the thyroid cells prevents

the appearance of MIT and DIT in the peripheral blood What purpose if any is served by this enzyme in other organs than the thyroid is not apparent In an assay for this enzyme in 39 glands from patients with familial goiter Carr et al found that the $K_m$ was normal except in the specimens from eight cancers of the thyroid where the $K_m$ was found to be low [66a]

## HORMONE TRANSPORT AND FATE

Thyroxine ($T_4$) is carried in the plasma in association with a carrier protein which has an electrophoretic mobility close to that of $\alpha_2$-globulin [67–69] In addition a small amount of $T_4$ is carried with albumin and recently a component has also been found in the prealbumin region in electrophoretograms of in vitro labeled plasma on hydrolyzed starch gel [70] and in Tris-maleate buffer [71] There is some question as to whether the inter α carrier protein and the prealbumin carrier may be one and the same and whether noted differences may be due to experimental conditions $T_3$ is also carried by the thyroid binding protein but it is less tightly bound and is found also in association with the other plasma proteins [72]

The equilibrium between free and bound iodothyronines may determine the amount of hormone which actually impinges upon cells to give the characteristic metabolic stimulating effect [69] The amount of effective or free hormone is a function of the amount of hormone in the blood and the amount of carrier protein

The thyroid hormones are degraded in the peripheral tissues through several pathways Small amounts appear as glucuronides in the bile [73] Isselbacher has found that these are formed by a liver microsomal enzyme which requires a uridine nucleotide [74] Deamination and decarboxylation products have been demonstrated in brain tissue [70] and Albright et al [76 77] have shown that the conversion of $T_4$ to $T_3$ may take place in kidney slices in vitro More recently they have also observed the conversion in isolated cell particulate preparations of liver kidney and other tissues [78] $T_3$ fails to appear in the serum in significant amounts after intravenous administration of $T_4$ Several other investigators have described slow deiodinization of $T_4$ by liver homogenates [79–82] Curiously enough rapid deiodination occurs in cell free and mitochondria free suspensions of liver tissue which have been heated to 100 C *before* addition of substrate [83] This degradation has been traced to a heat stable microsomal enzyme which requires oxygen and $Fe^{++}$ and is activated by cysteine and certain other organic reducing substances Whether this enzymatic system has any function in vivo remains to be seen Iodide released by degradation of iodothyronines is available for reentry into the iodide pool of the extracellular fluids Little if any iodothyronine appears in the urine and only small amounts in the feces

## PERIPHERAL ACTION OF THYROID HORMONES

It is not possible within the scope of this brief survey to examine in any detail the various theories which have been proposed to account for the stimulating effect of thyroid hormones on the oxygen consumption of cells An attempt will be made only to mention the leading directions of investigation and to cite a few introductory papers from the profuse literature on this complicated subject Only a few important contributions have been made since the excellent review of Wolff and Goldberg [11]

The peripheral action of thyromimetic substances is intimately related to structure but minimal structural requirements are not entirely determined An ether bridge between the two rings is necessary but can be replaced with a thioether The *p*-hydroxyl grouping can be replaced by a methoxy group and the alanine side chain can be radically altered Various substitutions of nitro amino alyl halogen and hydroxyl groups can be made for the iodines but almost always with diminution of activity [84 85] Removal of one iodine from the 3′ position enhances activity [86] The relationships of structure to function are further complicated by the fact that a structural alteration may have differing metabolic effects depending on which of several assays is employed This is not surprising when one considers that a structural change may alter absorption protein binding cell penetrance or degradation of the compound and that different assays may measure different effects of the substances in question

The iodothyronine which ultimately affects the machinery of the cell has not been identified The in vivo latency of several hours in the effect of thyroxine has suggested that it undergoes a structural alteration before it is active The shorter latency of $T_3$ and the still shorter one of the acetic acid analogue of $T_3$ indicate a possible pathway of metabolism toward the effective substance

**Effects on Peripheral Enzyme Activity** A large number of cellular enzymes of various tissues is influenced by thyroid administration [87] or thyrotoxicosis [87a] Succinic oxidase amino acid oxidases carbonic anhydrase ascorbic oxidase cytochrome oxidase arginase muscle hexokinase amylase and others vary with the thyroid dosage Lactic acid dehydrogenase and plasma pseudocholinesterase vary inversely Some of these effects may be the result of binding of metals by iodothyronines Thyroxine complexes with $Cu^{++}$ $Co^{++}$ $Zn^{++}$ $Mg^{++}$ $Fe^{++}$ and $Mn^{++}$

Thyroxine and several analogues inhibit a number of DPN-dependent dehydrogenases Among these are malic glutamic lactic triosephosphate yeast alcohol and yeast glucose 6-PO$_4$ dehydrogenases [88] All these enzymes contain zinc With the possible exception of malic dehydrogenase the effective concentration for $T_4$ effect is above the physiologic

range by two or three orders of magnitude. The inhibition of malic dehydrogenase accounts for the apparent stimulation of succinic oxidation by $T_4$, since succinic dehydrogenase is inhibited by oxalacetate which is formed by the malic dehydrogenase. This action of L-thyroxine on dehydrogenases is shared by the dextrorotatory isomer and by other substances which do not have thyroxine-like activity [89, 90]. Inhibition of DPN linked dehydrogenases has been attributed to a deleterious effect on mitochondria (which swell under the influence of thyroxine [91]), which permits leakage of DPN from these structures [92]. This may be one way in which dehydrogenase activity is inhibited, but Wolff and Wolff found the inhibition in a system which was free of particulate material [88].

Thyroxine binding of $Mg^{++}$ ion has been of particular interest because of the similarity of some of the effects of experimental magnesium deficiency to human thyrotoxicosis. Gershoff et al. [93] have found that the inhibition of growth of rats caused by administration of large amounts of $T_4$ can be at least partially overcome by feeding magnesium. The altered oxidative phosphorylation of heart muscle was also restored to normal.

It has recently been found that steroid metabolism is intimately related to thyroid function. Patients with thyrotoxicosis dispose of administered steroids such as cortisone more rapidly than normal, and those with myxedema less rapidly [94]. This has been traced to a control of capacity of the liver to reduce ring A of the steroid by $T_4$ [95]. Not only does treatment of rats with $T_4$ increase the availability in liver homogenate of TPNH for ring reduction, but with prolonged treatment there is an increase in the microsomal reducing enzyme as well [96]. Similar results have been obtained for testosterone, hydrocortisone, and others.

*Effects on Oxidative Phosphorylation*

Most of the chemical energy used by the body is channeled through high energy phosphate bonds, especially ATP. Oxygen is consumed in the production of this stored and readily available form of energy. The ratio of bonds formed (phosphate uptake) to oxygen used (P/O ratio) is thus a measure of the efficiency of oxygen utilization.

A significant advance in the understanding of thyroid hormones in relation to cellular physiology was the finding that there is a fall in the P/O ratio (uncoupling) of mitochondrial preparations in vitro when $T_4$ or a number of analogues is added, or when the P/O ratio is measured in tissue removed from animals pretreated with $T_4$. Many other chemical substances such as dinitrophenol also uncouple, as do optical isomers of L-thyroxine and many analogues and related substances [97–100].

The uncoupling explanation of $T_4$ action has been an attractive one. The principal arguments advanced against its acceptance are that (1)

many substances uncouple oxidative phosphorylation which are in no sense effective in the treatment of hypothyroidism (2) uncoupling fails to explain the normal state of thyroid function or the beneficial effect of $T_4$, (3) the concentrations of thyromimetic substances necessary for uncoupling are somewhat higher than those existing in vivo and (4) whereas $T_4$ may uncouple this takes place largely at the expense of phosphate bond formation but with little if any increase in oxygen consumption These objections have been largely answered by Lardy and Maley [97] who have proposed a mechanism for the central and most difficult problem namely, that of the specific beneficial effect of $T_4$ They propose that $T_4$ and analogues act at a specific biochemical locus when in low concentration to uncouple a rate-limiting step in the chain of energy yielding reactions In this way subsequent steps proceed more rapidly and without over all significant loss in efficiency Larger concentrations of $T_4$ permit the uncoupling effect to spread to other reactions and lower the over all efficiency It cannot be claimed that the objections have been entirely met until further evidence has accumulated but the mechanism of action proposed by Martius Lipmann and Lardy and their colleagues comes closer than any other to accounting for the facts

## CONTROL OF THYROID FUNCTION

The activity of the thyroid gland is largely under the control of the basophilic cells of the anterior lobe of the hypophysis which secrete thyrotropic hormone (TSH) Removal of the hypophysis reduces but does not entirely eliminate thyroid function [101] TSH has not yet been obtained in pure form and its mode of action on the thyroid is not entirely clear All measured functions of the gland are stimulated by TSH including both uptake of iodide and secretion of hormone [102]

The rate at which TSH is secreted depends upon the concentration of thyroid hormones in the circulating blood An increase in plasma concentration of $T_4$ causes a decrease in secretion of TSH and vice versa in a way provocatively analogous to feed back control of synthesis of enzymes in certain bacterial systems [103] Substances other than $T_4$ which have none of the beneficial effects of $T_4$ but which uncouple oxidative phosphorylation may also inhibit the secretion of TSH [104]

Thyrotropic secretion can also be influenced by the hypothalamus [105–107] The contribution of the hypothalamic center if any to normal *thyroid function* or to thyroid activity in disease states of the thyroid is by no means clear There is no question but that cold and other physiologic stimuli of thyroid function employ the hypothalamic pathway to produce their effects on the gland [108]

The concentration of $T_4$ in the plasma may exert a measure of control directly on the thyroid [109] Thyroid function and growth are influenced by the amount of $T_4$ given to hypophysectomized rats maintained on

constant doses of TSH The possibility that disposal of the TSH was influenced by the $T_4$ was not excluded in these experiments

It has already been pointed out that the rate of hormone synthesis in the thyroid is controlled in part by the amount of stored iodine within the gland [16 17] There is no satisfactory explanation for this mechanism of control, nor is it well understood how it is that large amounts of iodide inhibit secretion of thyroid hormones by the hyperfunctioning thyroid of Graves disease

## TYPES OF FAMILIAL GOITER

The preceding paragraphs have made it evident that there are many steps in the synthesis storage and secretion of the thyroid hormones Many, if not all, of these steps depend upon the functional integrity of specific enzymatic systems Conceivably, thyroid disease could arise from defective function of any one of these steps for should a step be blocked or ineffective production of thyroid hormones would be diminished or absent Compensatory growth and increased activity of the gland might or might not restore adequate formation of hormones

Several types of familial goiter are now recognized which can be classified with more or less certainty according to specific failure of a normal intracellular process The patients may be grouped as follows (1) those whose thyroids are unable to convert inorganic iodide into an organic form (2) a closely related group of patients who have a similar metabolic fault but who differ from the first group in severity and in ✓ having familial eighth nerve deafness (3) those who are unable to couple MIT and DIT into $T_3$ and $T_4$ (4) those who have a general tissue lack of iodotyrosine deiodinase (5) a group which may be heterogeneous and which is characterized by secretion of an abnormal iodinated peptide into the serum There are many reports of patients who doubtless belong in one or the other of these categories but who cannot be classified because of the meager data available Still other categories doubtless remain to be defined

### FAMILIAL GOITER FROM FAILURE TO FORM ORGANIC IODINE

*Case Studies*

Five cretins with goiter who share a common defect in organification of iodine have been studied in the author s laboratory [110-112] Four of these were siblings from a family of seven children Three older siblings were normal and without thyroid disease and now have a number of normal children The parents were half Caucasian and half American Indian and were first cousins on the Indian side of the family Both pairs of paternal grandparents were also first cousins Growth and de-

velopment of the affected children were reported normal during the first few months of life but thereafter became retarded Thyroid medication was begun in all between the ages of a few months and 2 years but was intermittent because of the impoverished state of the family A mass was observed in the neck of each between the ages of 7 and 13 and slowly increased in size until they were seen in the hospital

*Patient 1* was 16 years old in 1949 at the time of initial studies She was dwarfed and grossly retarded mentally The thyroid was enlarged five or six times normal size it was nodular and firm A bruit and thrill were present and the skin over the thyroid felt warm to touch The protein bound iodine (PBI) concentration of the blood was 1 02 μg per 100 ml

When the patient had been without thyroid medication for 3 weeks 100 microcuries of $I^{131}$ was given orally The labeled iodine in the gland reached a plateau in less than 2 hr Twenty seven hours later 1 0 gm of KSCN was given by mouth This caused an immediate and striking fall in the labeled iodine in the gland (Fig 9 2) At the end of 2 hr the counts had fallen to 25 per cent of the previous level

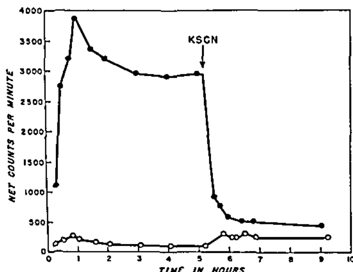



Fig 9-2 Discharge of iodide from the thyroid gland of a patient with cretinism and goiter caused by administration of thiocyanate $I^{131}$ was given at zero time Thiocyanate was given orally as indicated by the vertical arrow

At thyroidectomy the gland weighed 97 gm There was intense hyperplasia coupled with cystic degeneration and fibrosis (Fig 9-3) An analysis of the tissue by Douglas Riggs disclosed a concentration of thyroid inorganic iodide of 20 3 μg per 100 gm and of protein bound thyroid iodine of 10 4 μg per 100 gm The normal thyroid gland contains from 4 to 15 mg iodine of which 1 to 2 per cent is inorganic

One of the normal siblings of this patient was similarly studied Following a tracer of $I^{131}$ labeled iodine slowly accumulated in the thyroid and reached

constant doses of TSH The possibility that disposal of the TSH was influenced by the $T_4$ was not excluded in these experiments

It has already been pointed out that the rate of hormone synthesis in the thyroid is controlled in part by the amount of stored iodine within the gland [*16 17*] There is no satisfactory explanation for this mechanism of control, nor is it well understood how it is that large amounts of iodide inhibit secretion of thyroid hormones by the hyperfunctioning thyroid of Graves' disease

## TYPES OF FAMILIAL GOITER

The preceding paragraphs have made it evident that there are many steps in the synthesis storage and secretion of the thyroid hormones Many if not all of these steps depend upon the functional integrity of specific enzymatic systems Conceivably, thyroid disease could arise from defective function of any one of these steps, for should a step be blocked or ineffective production of thyroid hormones would be diminished or absent Compensatory growth and increased activity of the gland might or might not restore adequate formation of hormones

Several types of familial goiter are now recognized which can be classified with more or less certainty according to specific failure of a normal intracellular process The patients may be grouped as follows (1) those whose thyroids are unable to convert inorganic iodide into an organic form (2) a closely related group of patients who have a similar metabolic fault but who differ from the first group in severity and in having familial eighth nerve deafness (3) those who are unable to couple MIT and DIT into $T_3$ and $T_4$ (4) those who have a general tissue lack of iodotyrosine deiodinase (5) a group which may be heterogeneous and which is characterized by secretion of an abnormal iodinated peptide into the serum There are many reports of patients who doubtless belong in one or the other of these categories but who cannot be classified because of the meager data available Still other categories doubtless remain to be defined

### FAMILIAL GOITER FROM FAILURE TO FORM ORGANIC IODINE

*Case Studies*

Five cretins with goiter who share a common defect in organification of iodine have been studied in the author s laboratory [*110–112*] Four of these were siblings from a family of seven children Three older siblings were normal and without thyroid disease and now have a number of normal children The parents were half Caucasian and half American Indian and were first cousins on the Indian side of the family Both pairs of paternal grandparents were also first cousins Growth and de-

Two hundred microcuries $I^{131}$ was given intravenously Maximum accumulation was observed in the thyroid almost immediately The counting rate extrapolated to zero time corresponded to 68 per cent of the administered dose The quantity of the labeled iodine in the gland declined at a rate estimated at 2 5 per cent per hr (T ½ = 27 6 hr) for the first few days of observation and then appeared to approach an exponential fall at a rate of 1 9 per cent per hr (T ½ = 35 9 hr)



Fig 9-4 A 3 year-old cretinous male with goiter (Patient ? of text) (*By permission of The Journal of Clinical Endocrinology and Metabolism*)

The labeled iodine of the blood was measured frequently for the first few hours after administration and daily thereafter After a few hours the disappearance of labeled iodine from the serum followed a simple exponential having a rate constant of 1 9 per cent per hr The extrapolated concentration of iodide in the serum at zero time was 2 5 per cent per liter The volume of distribution was therefore 40 liters (100 per cent ÷ 2 5) Since 68 per cent of the administered dose was present in the gland at zero time it is evident that the inorganic iodide compartment of the thyroid accounted for 27 2 liters and that the extrathyroidal iodide compartment was 12.8 liters or 0 31 liters per kg body weight The last two values are within normal limits

a maximum value in approximately 24 hr Oral administration of 2 gm KSCN caused no discharge of labeled iodine from the gland

*Patient 2* a 23-year-old cretinous male sibling of Patient 1 was brought to the hospital because of a huge goiter and increasing difficulty in breathing (Fig 9-4) A PBI determination of the serum was zero

The patient was given a tracer dose of 100 microcuries of $I^{131}$ 5 months after he had last taken desiccated thyroid Four and one-half hours later

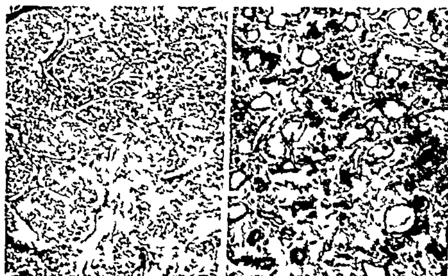


Fig 9-3 Histologic pattern from two areas of a thyroid gland removed from a patient with congenital goiter Note the hyperplasia

when the counts over the thyroid had reached a plateau he was given 2 gm KSCN by mouth There were an immediate rapid fall in the recorded counts over the gland and a rise in the radioactivity recorded over the lower back After 2 hr the counts in the neck had fallen by 70 per cent

*The removed thyroid weighed 497 gm Pathologic examination showed* findings similar to those in Patient 1 above In many areas there was a paucity of acinus formation characteristic of fetal adenoma and in others disorganization of cellular pattern and fibrosis The total inorganic iodine was 173 µg per 100 gm and the thyroid PBI was 15 µg per 100 gm

*Patient 3* a 13 year-old goitrous male sibling of the previous two patients was brought to the hospital for study His intellectual attainments were somewhat beyond those of his cretinous siblings He had learned to feed himself and to walk but had never gone to school

Labeled iodine accumulated rapidly in the thyroid during the first hour *after administration* and then slowly declined Two grams of KSCN orally caused an immediate discharge of the labeled iodine from the thyroid By 1 hr the counts were approximately 10 per cent of the previous level The concentration of PBI in the serum was 1 4 µg per 100 ml The excretion of iodine in the urine varied from 86 to 232 µg for 24 hr (normal)

This patient was studied again 4 years later The PBI concentration of the serum was 0 9 µg per 100 ml The thyroid gland was fully five times normal size and was multinodular

*Patient 5* a 13 year-old female was unrelated to the previous patients A diagnosis of cretinism was made at the age of 5 months and thyroid medication was given until several weeks before the studies described below were made Enlargement of the thyroid gland was noticed 1 year prior to admission Serum PBI concentration was 0 4 and 0 4 μg per 100 ml on successive analyses

Radioactive iodine accumulated rapidly in the region of the thyroid gland and reached a maximal value of 1 hr Two grams of KSCN was given by mouth Shortly thereafter there was a rapid disappearance of the labeled iodine from the region of the thyroid gland Subsequently the thyroid was removed The gland was composed of adenomas of varying degrees of cellular differentiation Some were solid masses of large thyroid cells in packed clusters and cords Others showed evidence of acinus formation with colloid production The degree of hyperplasia in many areas was intense and exaggerated The excised gland contained 0 62 μg total iodine per gram of wet tissue and 0 07 μg precipitable iodine per gram of wet tissue

**Similar Cases Reported by Others** There have been sporadic reports of other patients who appear to have the same pattern of iodide metabolism as that described above Schultz Flink Kennedy and Zieve [*113*] reported a 34 year old male cretin with a huge lobular thyroid gland Three cretinous siblings were not available for study Protein bound iodine concentration in the serum was 1 4 μg per 100 ml $I^{131}$ uptake reached maximum values at 1½ hr When 2 gm KSCN was given orally there were a precipitous fall in the labeled iodine of the thyroid and a rise in the $I^{131}$ concentration of the serum

Clayton Smith and Leiser [*114*] studied a family of six Negro children four of whom were goitrous The parents were not consanguineous nor did they have thyroid disease A maternal great aunt and uncle had goiter The goitrous members of this family differed from those already described in that they were euthyroid by clinical assessment and by laboratory study One had a PBI concentration above normal but a normal butanol-extractable iodine (BEI) Another of the goitrous siblings had an abnormally wide difference between the BEI and the PBI The four goitrous siblings all demonstrated a release of labeled iodine when given KSCN Studies on one of the normal siblings failed to show any effect of KSCN in causing release of labeled iodine but further uptake was inhibited Two goitrous siblings with small goiters showed definite but slight discharge of labeled iodine following administration of KSCN By chromatographic analysis $T_4$ and iodide were found in the peripheral serum and $T_3$ as well No MIT or DIT was found Histologic study of a biopsy specimen from one of these patients disclosed a variegated appearance Some areas showed intense hyperplasia whereas others showed colloid involution

Lelong et al [5] reported a 10 year-old retarded male with five normal siblings There was rapid accumulation of labeled iodine in the thyroid Two successive doses of KSCN of 1 gm each caused precipitous

Urine was collected in half hour fractions during the first few hours and thereafter in daily quantities. After the first few hours the daily excretion of labeled iodide fell along a simple exponential line closely paralleling those of the blood and urine. The proportion of gland content excreted in the urine per day was 5.15 per cent and was constant throughout.

The hypothesis that all the labeled iodine in the thyroid was in inorganic form was tested in several other ways in addition to the KSCN method. If *one assumed that the labeled iodine in the gland was in free exchange with* the iodide of the blood, then from the volume of distribution of the iodide and from the renal clearance of iodide it would be possible to predict the in vivo disappearance of labeled iodine from the gland. Renal clearance was measured on two separate occasions with six half hour collection periods. The mean value was 1.31 liters of plasma per hour. Since the volume of the iodide compartment was 40.0 liters, then 3.29 per cent of the iodide compartment was cleared per hour, corresponding to a half time of disappearance of 21.1 hr. The observed in vivo disappearance rate was estimated to have a half time of 27.6 hr during the initial few days of the experiment.

If it is assumed that the specific activity of iodine in the gland is the same as that in the blood and urine (an assumption which follows the hypothesis of the preceding paragraph), then from measurements of stable and labeled iodine in the urine it is possible to calculate the quantity of iodine present in the thyroid gland thus

$$Q = \frac{Q_e^{*}E}{E^{*}}$$

where $Q_e^{*}$ = the percentage of the administered dose of labeled iodine in the gland
$E$ = the 24 hr excretion of iodide
$E^{*}$ = the 24 hr excretion of labeled iodide

*The mean value of $Q_e$ calculated for 10 days was the astonishingly small* quantity of 257 μg.

The total iodine content of the gland was measured after surgery by chemical analysis of four fragments of a half gram each from four separate areas. Over 99 per cent of the iodine was trichloracetic acid–soluble. The calculated iodine content proved to be 319 μg, a figure which agrees well with the calculated estimate of iodine content of the whole gland based on the hypothesis that all the iodine contained therein was in inorganic form. Chromatographic analysis of the four fragments of tissue also failed to demonstrate any organically bound labeled iodine.

*Patient 4*, the oldest cretinous sibling of the previous patients, was first admitted to the hospital at age 13 for a mass in his neck. Two hundred and thirty five grams of thyroid tissue was removed. The histologic appearance was similar to that described for the three siblings. The patient was readmitted to the hospital at age 27 because of regrowth of the goiter and pressure symptoms causing respiratory difficulty. An emergency tracheotomy was required and this was followed by a total thyroidectomy. Approximately 175 gm thyroid tissue was removed. In addition to the same histologic pattern which was evident at the time of the earlier thyroidectomy there was an area of vascular and capsular invasion which was considered to be diagnostic of malignant change. There has been no evidence of recurrent disease in the 2 years of follow up. No other laboratory investigations have been made on this patient.

None was observed although normal human thyroid tissue under identical conditions readily formed MIT The authors preferred the possibility that the gland had an iodinase defect rather than defective formation of active iodide or that there might be an abnormal receptor protein

*Genetics*

Too few families with this disorder have been reported at the present time for a firm interpretation of the genetics of this disease If one excluded the cases of Clayton et al on the grounds that their patients were not cretinous then 10 affected patients have been reported in 4 families of 28 children The sex of 3 of the affected persons is not given but 2 are female and 5 are male Although in the patients reported to date there is a slight excess of affected sibling above the expected ratio the data are not inconsistent with inheritance as a simple autosomal recessive gene This interpretation is also consistent with the consanguinity in the family of Patients 1 to 4

The genetic interpretation of Clayton s patients who were not hypothyroid is difficult It seems unlikely that they could be heterozygous for the same gene for which other members of this group are homozygous because parents of other sibships have not shown evidence of thyroid disease and yet must have been heterozygotes As already mentioned it is possible that oxidation of iodide to iodine and fixation to tyrosyl residues form a complex rather than a single-step process and that the response to thiocyanate might be similar in patients having different defects in hormone synthesis

## FAMILIAL GOITER AND DEAF MUTISM

*Case Studies*

Since Brain reported 6 families of congenital goiter and deafness in 1927 [118] at least 12 other families have been reported with this association of diseases With few exceptions the clinical pattern has been uniform Goiter has been apparent either at birth or within the first decade of life and nerve deafness has been present from birth or has developed during childhood Intelligence and usually growth have been normal Vestibular function has been normal or nearly so and the tympanic membranes have been intact

These patients have not had huge goiters Thyroid function as assessed by the usual clinical and laboratory data has been normal in most of them The serum concentrations of PBI in most of the affected persons of Johnsen s three kindred were distinctly low [119] Two of the four affected members of the sibship of Elman [120] had developed invasive adenocarcinoma and Roberts case [121] had localized histologic change

but partial falls of the labeled iodine in the gland from the maximal uptake of 57 per cent Three similar patients have been reported by Gardner et al [115]

A 13 year-old cretin briefly mentioned by Federman et al [116] had a PBI of 0 7 μg per 100 ml and a bone age of 1 year Maximal uptake of $I^{131}$ was 30 per cent at 4 hr and 90 per cent of this was discharged from the gland upon administration of KSCN There was a concurrent rise of labeled iodine in the blood as recorded over the thigh The cretinous infant described by Jackson [117] may also belong to this group but thiocyanate caused a release from the gland of only approximately 30 per cent A control study without KSCN showed almost complete loss of labeled iodine from the gland in 48 hr

*Physiologic Interpretation*

The common denominator of the patients in this group is the release of labeled iodine from the thyroid gland upon the administration of KSCN The clinical findings have not been uniform The majority have been severe cretins with profound retardation of structural and intellectual development Those reported by Clayton et al [114] were clinically euthyroid as confirmed by laboratory examination

The release of labeled iodine from the thyroid by thiocyanate indicates a store of inorganic iodide in the gland which can be readily displaced Normally virtually none of the accumulated iodine can be displaced in this way Those patients who showed a thiocyanate release of labeled iodine appear to have a metabolic defect which makes them unable to convert accumulated iodide to organically bound iodine Since this limits or prevents hormone synthesis compensatory hypertrophy and hyperplasia of the thyroid occur as do the consequences of an insufficient hormonal supply Support of this interpretation has come from chromatographic and analytic studies The kinetics of iodine metabolism reported from Patient 3 above were consistent with a large iodide space in the thyroid in free communication with the iodide space in the blood and inconsistent with any store of bound iodine in the thyroid gland Tissue analyses on three of the patients in that kindred indicated virtually no organically bound iodine in the gland

The biochemical lesion in the thyroid of these patients seems established inferentially but not precisely or directly Unquestionably there is interference or failure in the step where iodide becomes oxidized to iodine and displaces a proton from a tyrosyl residue The enzyme or enzymes which may be involved have been discussed earlier The evidence is in accord with an absence of the specific oxidative enzymatic process in the thyroid gland which is responsible for conversion of iodide ion to $I_2$ (or HIO) Haddad and Sidbury [114a] have had the opportunity to measure the capacity of a gland from a similar patient to form MIT

[120a] Similar findings were obtained in one similar patient by Buchanan and Crooks [123a] In their second patient there was no discharge after administration of perchlorate but in spite of a 75 per cent uptake of $I^{131}$ by the thyroid no $PBI^{131}$ was found and the labeled iodine was largely gone from the gland at the end of 24 hr

Among the reported patients in this group no members of the families have goiter without deafness or the reverse The sex distribution is nearly equal Morgans and Trotter pointed out that this is unusual since ordinarily thyroid disease is much more common in females than in males Only in Johnsen s kindreds has the disease appeared in successive generations (Fig 9-5)

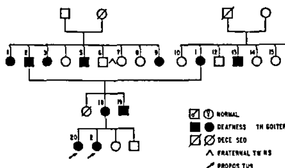



Fig 9-5 Inheritance of deafness and goiter in an intermarried family studied by Johnson [119] (*Redrawn and printed by permission from Acta oto-laryngologica*)

The inheritance of this disease is not entirely clear from the family charts available Approximately one-half the sibs in the reported families have been afflicted Although the pattern is not entirely inconsistent with an autosomal dominant form of inheritance it is probably best at the moment to withhold judgment It is by no means clear why the thyroid and the eighth nerve are both involved Close linkage of the relevant genes could obtain or both goiter and deafness could be the result of pleiotropic action of a single gene

## CRETINISM WITH FAILURE OF CONDENSATION OF IODOTYROSINES

### *Case Study*

Defective coupling of MIT and DIT into $T_3$ and $T_4$ was the cause of hypothyroidism and goiter in a 25-year-old retarded female studied in the author s laboratory [11 ] A diagnosis of hypothyroidism was made at age 4 desiccated thyroid was prescribed but taken only intermittently A goiter was first observed at age 17 At 2? the serum concentration of PBI was 3 5 µg per 100 ml The daily excretion of $I^{127}$ was normal at a time when the patient had ingested no desiccated thyroid for the previous 8 weeks Measurements of the basal metabolic rate and PBI were normal at the time of

suggestive of malignant degeneration Two of the four patients in Thieme s sibship also had local malignant degeneration without evidence of distant extension [122]

*Thyroid Function Studies*

Laboratory observations designed to clarify the thyroid function of these patients have been performed principally by Morgans and Trotter [123] by Elman [120] and by McGirr and Hutchison [120a] Morgans and Trotter gave radioactive iodide to two patients and made serial measurements of labeled iodine over the neck and thigh An identical study was done on the normal mother of these two siblings One hour after administration of radioiodine each of the two patients received 200 mg of $KClO_4$ in water by mouth Within the next 30 min there was a *fall of approximately 30 per cent in the counting rates recorded over the* neck and a rise over the thigh The administration of perchlorate to the mother of the two patients was attended by little change either in neck or thigh counting rates It was concluded from these observations that these patients had a partial defect in the organification of labeled iodine Similar results and interpretations derive from the studies of McGirr and Hutchison [120a]

The studies of Elman were more limited in scope He observed that in one of his patients the $I^{131}$ uptake in the neck was 30 per cent in 1 hr and 23 per cent in 24 hr In the other patient the respective figures were 20 and 16 per cent He drew no conclusions from these observations other than to suggest that the fall in labeled iodine of the thyroid between the first and twenty fourth hours indicated some sort of enzymatic abnormality in the thyroid gland Further and more detailed studies of similar patients should be rewarding

*Genetics*

Morgans and Trotter [123] summarized the literature until 1958 and found 20 affected siblings among sibships totaling 41 Johnsen s 2 families included 24 members of whom 21 were personally examined [119] (Fig 9 5) Of these 12 exhibited goiter and deafness Four of his cases were the result of intermarriage of 2 affected members of the 2 families Four of 7 siblings of Elman s sibship were affected Deraemacker [124] in Belgium found 3 affected males in a sibship of 12 from a first cousin marriage Interestingly 2 of these had associated icthyosis The author excluded a chance association of recessive genes (or genetic defect) on statistic grounds Perhaps with less justification he excluded linkage and concluded that the disease was to be understood as an instance of a pleiotropic gene effect of a recessive gene Discharge of approximately 50 per cent of accumulated $I^{131}$ was caused by oral thiocyanate or perchlorate to all five of the recently reported patients of McGirr et al

Chromatographic analysis of serial serum samples taken between the seventh and seventy ninth hours after administration of $I^{131}$ disclosed $T_4$ and $T_3$ in all samples Yet in spite of heavy labeling of the gland and rapid turnover of iodine by the gland it was impossible to demonstrate either $T_4$ or $T_3$ in four tissue specimens obtained at operation although iodide and abundant MIT and DIT were demonstrated with ease The specimens were obtained 79 hr after the administration of $I^{131}$ One hundred and thirty grams of tissue were homogenized hydrolyzed and analyzed for $T_4$ by the method of Gross and Pitt Rivers [*195*] Pitt-Rivers was unable to demonstrate more than a trace of $T_4$

### *Physiologic Interpretation*

Two lines of investigation indicated that the primary fault in the thyroid gland of this patient was an inability to couple MIT and DIT into $T_3$ and $T_4$ The more significant evidence derived from chemical studies on the blood and on the excised thyroid gland Serial chromatographic analyses of the serum for many hours after the administration of $I^{131}$ demonstrated the early appearance of $T_4$ and of $T_3$ but of no unidentified iodinated substance Detailed analysis of the glandular tissue failed to disclose labeled $T_4$ or $T_3$ Only when the whole gland was subjected to chemical analysis was it possible to identify a trace of $T_4$ Yet at the same time abundant quantities of MIT and DIT were found In the normal thyroid gland at least 20 per cent if not more of the labeled iodine is in the form of labeled $T_4$ by [illegible]9 hr Thus it appeared that while this hyperplastic gland was making ample MIT and DIT and was storing them in large amounts in the gland the $T_4$ and $T_3$ which were produced were immediately released into the blood There was evidence therefore of an abundant formation of hormone precursors in the form of iodotyrosines but of limited formation of iodothyronines

Supporting information was obtained from kinetic studies of iodine metabolism in this patient For the details of these observations and their interpretation the reader may consult the original publication [*112*] It was possible to measure the rate at which iodine was leaving this gland in two ways During administration of methimazole the specific activity of the labeled iodine which appeared in the urine was extremely high This proved to be 1[illegible] per cent per mg at a time when the specific activity of the whole gland was only [illegible] 9 per cent per mg This finding could only be interpreted as indicating that methimazole was blocking the reutilization of labeled iodine derived from a pool of high specific activity whereas most of the labeled iodine in the thyroid was in a pool of low specific activity

The amount of unlabeled iodine leaving the gland daily and coming into equilibrium with the extrathyroidal iodine compartments was estimated from the equilibrium expression

$$H = \frac{Et}{1 - t}$$

where $H$ = the quantity leaving the gland
$E$ = the daily excretion of $I^{131}$
$t$ = the fractional uptake

Although $H$ could not be estimated precisely because of the sensitivity of the expression to small changes in $t$ when $t$ is close to 1 as it was in this patient still the calculated value of $H$ was at least 1 mg The normal

study. A total thyroidectomy was performed at the completion of studies. Microscopically the specimen displayed extreme hyperplasia.

The family history was of particular interest because it displayed two first-cousin marriages (Fig. 9-6) in the immediate antecedents of the patient and because a sister 4 years older suffered from an identical disorder. Daily excretion of $I^{127}$ was normal on several occasions. Concentration of PBI in the serum was 6.1 µg per 100 ml. She had undergone an uneventful subtotal thyroidectomy because of rapid growth of the gland. Pathologic examination disclosed multinodular goiter with extreme hyperplasia and almost no colloid formation within the follicles. Earlier studies on both sisters had shown an unusually rapid uptake of $I^{131}$ to values in excess of 95 per cent. The labeled iodine in the thyroid was not dislodged from either patient by administration of KSCN.

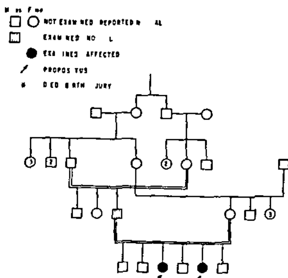



Fig. 9-6. Inheritance pattern of a family with two siblings with congenital goiter (see text). Note the two consanguineous marriages.

The special studies performed included detailed examination of the metabolism of iodine by measurements of the uptake, retention and release of labeled iodine and the excretion of stable iodine, and by chemical examination of the histologic specimen at the time of surgery. Labeled iodine accumulated rapidly in the thyroid to a maximum of 99.3 per cent within the first 2 hr. Thereafter it was released at a rate approaching 3.0 per cent per day. When methimazole was given there was a sharp increase to 7.3 per cent per day. When methimazole was withdrawn, the release rate slowed. The iodide clearance by the kidney was 30.5 ml per min. The renal excretion rate of labeled iodine rose sharply upon the administration of methimazole; also the daily excretion of stable iodine rose from normal values to a mean daily $I^{127}$ excretion of 203 µg per day above mean control values. The labeled iodine in the plasma fell sharply immediately after administration of $I^{131}$ but began to rise after a few hours and reached a maximum on the fifth day of 0.52 per cent per liter and then slowly fell

Chromatographic analysis of serial serum samples taken between the seventh and seventy ninth hours after administration of $I^{131}$ disclosed $T_4$ and $T_3$ in all samples Yet in spite of heavy labeling of the gland and rapid turnover of iodine by the gland it was impossible to demonstrate either $T_4$ or $T_3$ in four tissue specimens obtained at operation although iodide and abundant MIT and DIT were demonstrated with ease The specimens were obtained 79 hr after the administration of $I^{131}$ One hundred and thirty grams of tissue were homogenized hydrolyzed and analyzed for $T_4$ by the method of Gro s and Pitt Rivers [145] Pitt-Rivers was unable to demonstrate more than a trace of $T_4$

## *Physiologic Interpretation*

Two lines of inve tigation indicated that the primary fault in the thyroid gland of this patient was an inability to couple MIT and DIT into $T_3$ and T The more significant evidence derived from chemical studies on the blood and on the excised thyroid gland Serial chromatographic analyses of the serum for many hours after the administration of $I^{131}$ demonstrated the early appearance of $T_4$ and of $T_3$ but of no unidentified iodinated substance Detailed analysis of the glandular tissue failed to disclo e labeled $T_4$ or $T_3$ Only when the whole gland was subjected to chemical analysis was it possible to identify a trace of $T_4$ Yet at the same time abundant quantities of MIT and DIT were found In the normal thyroid gland at least 20 per cent if not more of the labeled iodine is in the form of labeled T by 79 hr Thus it appeared that while this hyperplastic gland was making ample MIT and DIT and was storing them in large amounts in the gland the $T_4$ and $T_3$ which were produced were immediately released into the blood There was evidence therefore of an abundant formation of hormone precursors in the form of iodotyrosines but of limited formation of iodothyronines

Supporting information was obtained from kinetic studies of iodine metabolism in this patient For the details of these observations and their interpretation the reader may consult the original publication [112] It was possible to measure the rate at which iodine was leaving this gland in two ways During administration of methimazole the specific activity of the labeled iodine which appeared in the urine was extremely high This proved to be 17 per cent per mg at a time when the specific activity of the whole gland was only 2 9 per cent per mg This finding could only be interpreted as indicating that methimazole was blocking the reutilization of labeled iodine derived from a pool of high specific activity whereas mo t of the labeled iodine in the thyroid was in a pool of low pecific activity

The amount of unlabeled iodine leaving the gland daily and coming into equilibrium with the extrathyroidal iodine compartments was estimated from the equilibrium expression

$$H = \frac{EU}{1 - U}$$

where $H$ = the quantity leaving the gland
$E$ = the daily excretion of $I^{127}$
$U$ = the fractional uptake

Although $H$ could not be estimated precisely because of the sensitivity of the expre ion to small changes in $U$ when $U$ is close to 1 as it was in this patient still the calculated value of $H$ was at least 1 mg The normal

amount of hormonal iodine secreted per day lies between 50 and 150 μg Only a small fraction of the iodine leaving the gland could have been in the form of thyroid hormone because otherwise the patient would have been thyrotoxic Accordingly the thyroid must have been releasing iodide or a readily metabolized iodinated substance which was quickly broken down either by the gland itself or in the periphery to yield iodide No other interpretation seems to fit the data

*The simplest formulation to account for the facts was that the coupling* of DIT to yield $T_4$ took place only to a limited degree perhaps because of partial or total deficiency of a coupling enzyme The $T_4$ which was formed was secreted rapidly perhaps without being staged through thyroglobulin In this way it is possible to account for the almost complete absence of $T_4$ from the gland despite normal concentrations in the peripheral blood The highly stimulated gland maintained an exceedingly rapid flux of some of its iodine Some went on to form hormone some was retained for storage as MIT and DIT and a large fraction—perhaps in the form of MIT—was *formed and released for deiodinization either in the gland or in the periphery* or both

It has not been possible to demonstrate a defect in $T_4$ coupling directly because the precise biochemical pathway by which this takes place has not been defined That the disease occurred in two sisters studied by the author strongly suggests a common biochemical defect under genetic control

## *Similar Cases Reported by Others*

Two unrelated female patients were carefully studied by Mosier Blizzard and Wilkins *[196]* One was 13 and the other 37 Both were hypothyroid and retarded and both had large goiters Serum concentration of protein bound iodine were low Thyroidal uptake of $I^{131}$ was rapid in both and peak values were reached within 3 hr The uptake was normal in the younger patient and low in the older Administration of KSCN caused little change in the labeled iodine in the gland No abnormal iodinated substances appeared in serum or urine after administration of $I^{131}$ There appeared to be diminished deiodination of intravenously administered labeled DIT when the patients were hypothyroid but this was restored to normal when they *were receiving full doses of desiccated thyroid Homogenates of the surgi*cally removed glands deiodinated DIT normally

The significant findings were in the glands Chromatographic analysis of the tissue disclosed that the labeled iodine was in the form of MIT and DIT Almost none was present as labeled $T_4$ although T had been demonstrated in the peripheral blood Although only 24 and 30 hr elapsed between administration of $I^{131}$ and surgical removal of the two glands by this time much more of the labeled iodine would normally have been in the form of $T_4$ than was in fact found Mosier et al concluded that the metabolic defect in these two patients was a block in the coupling of iodotyrosines into $T_3$ and $T_4$

Similar patients have been described by Joseph and Job *[127]* Two females from a sibship of 10 were hypothyroid and had goiter Each patient had a high uptake of $I^{131}$ and prolonged retention in the gland There was no discharge from the gland upon the administration of $SCN^-$ When $I^{131}$ labeled DIT was administered intravenously the principal labeled iodine which appeared in the urine was inorganic in form Chromatographic analysis of a biopsy specimen from the thyroid of one of these patients disclosed

that 60 to 70 per cent of the labeled iodine was in the form of DIT and 21 to 25 per cent in the form of MIT The rest of the iodine was present as iodide and if $T_4$ or other iodothyronines were present they were in a concentration of less than 1 per cent of the total

A puzzling case which may belong in this category has been reported by Werner et al [128] Their patient was a 5 year old female without retarded development Uptake of $I^{131}$ by the large goiter was repeatedly found to be elevated and turnover by the gland was rapid The serum concentration of PBI was 28 4 µg per 100 ml Labeled MIT DIT $T_3$ and $T_4$ were identified in the serum by the chromatographic methods employed but degradation of intravenously administered labeled DIT was normal and no abnormal iodinated substances were found in the urine Analysis of the gland removed 24 hr after administration of $I^{131}$ disclosed MIT and DIT but only small quantities of iodothyronines Werner et al concluded that their patient suffered from a defect in MIT and DIT coupling with subsequent spillage of these into the blood An 8½ year-old female with certain similarities has been reported in abstract by Whitelaw et al [129] Studies of other patients in this category have shown neither the iodotyrosines nor the remarkable elevation in serum concentration of PBI

The patients of Joseph and Job and of Mosier et al correspond closely in most details with those from the author s laboratory The author s patient and her sister had achieved a euthyroid state but earlier clinical observations had indicated clearly that they had been hypothyroid There are numerous reports in the literature of patients who conform to the pattern of these patients clinically and who also have rapid and high uptake levels of radioactive iodine [130–134] The cretinous brothers studied by Lerman et al [130] were of particular interest because they were the first to be studied physiologically and because of the intense hyperplasia which the glands displayed They are well and free of goiter at the present writing

Until simpler and more reliable methods are developed for defining patients of this group at a molecular level it seems unlikely that many can be placed with certainty in this category At the present time the minimal criterion for inclusion depends upon chromatographic analysis of the labeled iodine in the thyroid removed more than 3 days after administration of the labeled iodine Labeled iodothyronines are found at most in trace amounts whereas labeled iodotyrosines are present in abundance A word of caution is in order here large sluggish colloid filled glands may form $T_3$ and $T_4$ very slowly from MIT and DIT

*Genetics*

Little can be said regarding the genetics of this group The author s 2 patients had no family history of thyroid disease except for their own sibship but there were two first cousin marriages among their antecedents The family described by Joseph and Job [127] with 2 afflicted

members out of 10 in the sibship suggests a simple recessive gene All 6 of the patients who can be accepted at present are female

## CRETINISM FROM FAILURE OF IODOTYROSINE DEIODINASE ACTIVITY

### *Case Studies*

In 1953 Hutchison and McGirr [*155*] reported 12 patients from the Royal Hospital for Sick Children and the Royal Infirmary in Glasgow Scotland who were cretinous and hypothyroid Four of these were siblings and 3 and 1 subsequently described [*156*] were from the same kindred These 8 patients were of itinerant tinker stock an isolated group of people living a nomadic existence in Argylshire and the Isle of Selay Two others of the cretins were half-siblings and were also of tinker stock but from Perthshire and Angus

The tinker cretins were all goitrous The uptake of $I^{131}$ of all but 2 was high and the maximum value was quickly reached Most of the glands lost a large fraction of the accumulated labeled iodine within a few hours after maximum uptake Only 2 failed to show a high concentration of $PBI^{131}$ in the peripheral blood at 48 hr Analyses of the serum of 3 of these patients disclosed an organic butanol-extractable substance This component was not identified but the possibility was entertained that it might be DIT Chromatographic studies of the serum of 1 patient failed to identify the component as an iodothyronine

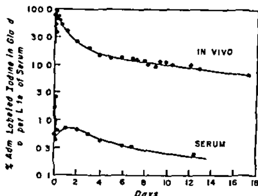



Fig 9-7 Metabolism of iodine in Patient A I was given orally at zero time and serial counts made over the thyroid and in the serum

Three similar patients were studied at the Academisch Ziekenhuis in Leiden in 1955 [*137 138*] The first of these was a 27 year-old male (Patient A) who was found to have a goiter at birth Thyroid medication was given for many years but was discontinued 4 years prior to entry to the hospital because of tuberculosis Since that time the goiter had increased relentlessly in size The serum concentration of PBI was 0 5 $\mu g$ per 100 ml His parents were not consanguineous and were normal as was an older brother but a younger brother (Patient B) who was also available for study had a goiter at birth and was retarded both physically and mentally A third unrelated patient (Patient C) was a 12 year-old female cretin with a huge multinodular goiter short stature and mental deficiency

The initial study of Patient A concerned the fate of an administered dose of $I^{131}$ [*137*] Labeled iodine accumulated unusually rapidly in the thyroid (Fig 9 7) Within 70 min 74 per cent had appeared in the neck region and during this time there was a rapid fall in the serum concentration of labeled iodine After 70 min the labeled iodine began to leave the thyroid and to appear in the blood as a component which was partially precipitable with trichloracetic acid By 48 hr only 25 per cent of the administered dose remained in the gland The serum concentration of labeled iodine had risen

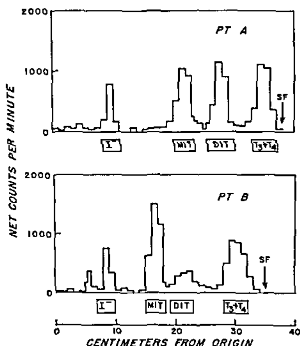



Fig 9-8 The labeled substances appearing in the blood of t o brothers with congenital goiter f llowing adm nistration of I The chromatograms were developed in the butanol-acetic acid solvent system The block symbols rep esent the position on tl e chromatogram taken by the added markers Note particularly the presence of i linated amino acids in the serum

to 0 5 per cent per liter and from that point it slowly fell during succeeding days Nearly identical in vivo retention and release curves were obtained from Patients B and C [*138*]

Specimens of serum obtained at 2 4 8 16 and 22 hr were extracted with butanol and chromatographed in a butanol dioxane-ammonia solvent system In successive specimens there were increasing amounts of labeled iodine which had the chromatographic mobility of MIT and DIT Moderate amounts of $T_4$ and $T_3$ were al o found Confirmation of the pre ence of MIT and DIT was obtained in a butanol-acetic acid solvent system The largest fraction of the labeled iodine in the serum was present as these two components (Fig 9 8)

The gland was removed because of pressure symptoms The histologic pattern was that of intense hyperplasia but some areas showed a tendency to form colloid and there were also areas of cystic degeneration and fibrosis The gland weighed 198 gm at operation Three and four tenths per cent of the labeled iodine was recovered as $T_4$ The remainder was found in small amounts as iodine and in large amounts as MIT and DIT

The blood findings on Patient A were duplicated in his brother (Patient B) After administration of $I^{131}$ large amounts of labeled iodine which was not inorganic in form appeared in the serum Several iodinated components were present on chromatograms of successive urine specimens (Fig 9-9) One of these appeared between the origin and the iodide zone in the butanol–acetic acid solvent system Another traveled near the solvent front In addition labeled iodine was present at the MIT and DIT zones The component near the origin was readily hydrolyzed into MIT by treatment with dilute acid but the conjugated substance was not positively identified

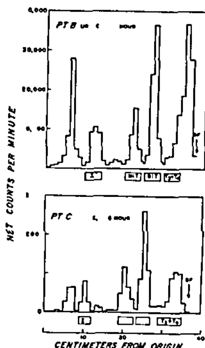



Fig 9-9 The labeled compounds appearing in the urine following oral administration of $I^{131}$ to two patients with cretinism and goiter Note the presence of iodinated amino acids large quantities of labeled substances moving near the solvent front and a large zone of labeled iodine near the origin The last was hydrolyzed with HCl to MIT

Observations on Patient C were more limited in scope The in vivo thyroidal uptake reached 70 per cent in 1 hr and then fell rapidly The same iodinated components in approximately the same proportion were found in successive urine specimens as were found in specimens from Patient B (Fig 9-9)

Subsequently the fate of injected labeled MIT and DIT was observed in Patients A B and C and in several relatives of Patient C In preparation for these studies the normal metabolism of labeled MIT and DIT was studied in a group of 15 subjects and the fate of intravenously administered L-MIT was studied in three subjects without thyroid disease [*139*] The results confirmed the earlier studies of Albert and Keating [*140*] who found that after intravenous administration of $I^{131}$ labeled DIT only a small fraction of the labeled iodine is excreted in the urine as free iodide Labeled DIT is deiodinated in vivo and labeled iodide increased in the serum *relative* to labeled DIT Within an hour after administration the relative amount of labeled iodine of the serum *as DIT* fell from approximately 90 per cent to approximately 65 per cent The labeled iodine was excreted in the urine largely as inorganic iodide (Fig 9 10) During the first few hours after administration the largest percentage of the administered dose which ap-

peared in the urine of any patient as DIT was 7 3 and most of the values were below 3 The only patient with a value above 3 per cent was one with diabetes mellitus and glycosuria

The results of experiments with labeled L-MIT in normal subjects were similar excepting that scarcely any detectable MIT appeared in the urine A small zone of labeled iodine was found between the iodide zone and the

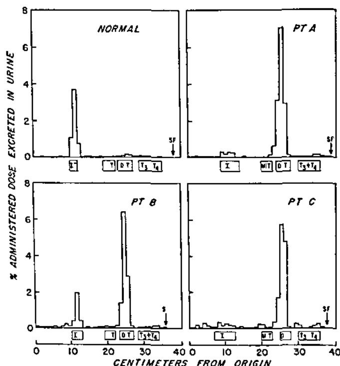



Fig 9 10 Chromatograms of urine after intravenous administration of labeled DIT to a normal person and to Patients A B and C The administered DIT appears as iodide in the normal person and almost entirely as DIT in the three patients

origin in chromatograms which were developed in the butanol–acetic acid solvent system When the racemic form of labeled MIT was administered a moderate amount of labeled iodine appeared near the solvent front and a large fraction was recovered in the urine as MIT Thus it appeared that both L-MIT and L-DIT were rapidly degraded upon intravenous administration and that the labeled iodine which appeared in the urine was almost entirely in the form of inorganic iodide

$I^{131}$ labeled DIT was given intravenously to Patients A B and C The results were the same in all three (Fig 9 10) Almost all the injected labeled iodine was excreted within the first 24 hr and almost all of it was excreted *as diiodotyrosine* There was no evidence that any of the DIT was deiodinated by any one of these three patients and there was little evidence of conjugation or deamination A small and questionably significant amount

of labeled iodine was found near the solvent front In the first 24 hr after administration 78 6 78 1 and 66 2 per cent of the injected labeled DIT was recovered *unchanged* in the urine of Patients A B and C

These patients also were given DL-MIT intravenously In contrast to normal individuals the patients showed practically no excretion of labeled iodide Almost all the labeled iodine appeared in the urine either as MIT or as zones of labeled iodine near the solvent front or near the origin in the butanol-acetic acid solvent system

Limited but similar studies were permitted on the sister mother two maternal aunts and one maternal cousin of Patient C The sister age 20 was entirely normal except for a moderately firm enlargement of her thyroid gland to about twice normal size The mother of Patient C age 43 had a subtotal thyroidectomy at 27 for progressive thyroid enlargement but there was recurrent growth of the gland to about five times normal size at the time of study The patient otherwise was normal and her serum concentration of PBI was normal One of the maternal aunts had a thyroidectomy for an enlarged thyroid gland which had been diagnosed as Graves disease The other maternal aunt was said to have had a goiter in the past but this had disappeared One of her daughters (cousin of Patient C) had a surgical thyroidectomy at age 20 and had a recurrent goiter of about four times normal size at the time of study A sibling (cousin of Patient C) had died at age 9 on the day prior to a scheduled thyroidectomy for Basedow s disease In each of the five members of the kindred a dose of labeled DIT was given and the excretion rate measured during the subsequent 4 hr The results were compared with those obtained from the 15 patients without thyroid disease discussed earlier It was found that the mean excretion rate of unchanged labeled DIT was significantly higher than in the control group although a large fraction of the DIT was deiodinated (Table 9-1)

TABLE 9-1 EXCRETION RATE OF UNCHANGED LABELED DIT

| Subjects | Per cent injected dose DIT excreted as DIT | | |
|---|---|---|---|
| | 0–1 hr | 0–2 hr | 0–4 hr |
| 15 control patients | 1 8 | – 4 | 2 8 |
| 3 patients with congenital goiter | 2) 2 | 4( 2 | (2 3 |
| 5 relatives of one of above patients | 4 6 | 6 2 | 7 1 |

When it became apparent that the thyroid of Patient B would be removed because of pressure symptoms the capacity of beef thyroid tissue to deiodinate labeled DIT was studied The method employed was that of Roche et al [63] Tissue slices were incubated for 6 hr in the presence of $I^{131}$ labeled L-DIT Approximately 60 per cent of the substrate was deiodinated Similar findings were obtained with slices from human thyroids received at the time of surgical removal because of nodular goiter [141]

The thyroid gland of Patient B then became available On the day of operation a nodular goiter from another patient was also available from the operating room Identical experiments were set up to demonstrate the deiodinating capacity of tissue slices from these two glands A normal amount of deiodination was demonstrated in the nodular goiter but the

thyroid tissue slices from Patient B failed to deiodinate the substrate DIT [141]

Detailed analytical studies of two of the tinker cretins of McGirr and Hutchison [135 136] have clearly shown that they have the same type of thyroid disorder as Patients A B and C Chromatograms of serum samples of one of their patients disclosed MIT and DIT as well as iodothyronines MIT DIT and two unidentified zones of labeled iodine were found in chromatograms from both urine specimens [142 143] McGirr and Hutchison have pointed out quite rightly that the disorder is the result of a single genetic defect and deserves to be categorized after Garrod as another inborn error of metabolism [136]

Almost simultaneously with the publication of the studies on Patients A B and C Horst [144] published a brief account of his observations on two goitrous cretins MIT and DIT were identified in serum samples Horst offered the same interpretation of his patients as for those already described [138]

Costa et al [145] have chromatographic evidence of DIT in the serum of three cretins from a region of endemic goiter in northern Italy Patients with endemic goiter do not customarily have MIT or DIT in blood or urine The in vivo curves obtained on two unrelated retarded goitrous subjects by Bernheim and Berger [146] are similar to those on Patients A B and C Burrell and Gardener [147] suggested that their three cretinous siblings (one with goiter) might be secreting DIT The thyroids of their patients had strong avidity for $I^{131}$ and the serum concentration of $PBI^{131}$ was much elevated at 48 hr

*Physiologic Interpretations*

Patients of this category share a defect in their capacity to deiodinate MIT and DIT The deiodinating enzyme has been described normally not only in the thyroid but in liver kidney and other organs The metabolic defect is both intra and extrathyroidal for not only are the afflicted patients unable to deiodinate MIT and DIT in their thyroid glands but when these substances are administered either before or after thyroidectomy or to the patient receiving full doses of desiccated thyroid the labeled iodotyrosine appears almost quantitatively in an unchanged form in the urine or in the case of MIT as conjugates

An objection has been raised that the hypothesis of a single enzymatic defect in these patients is insufficient to account for the poor production of $T_4$ and for the hypothyroidism [6] It has been suggested that there must also be an associated defect in the coupling of iodotyrosines into $T_3$ and $T_4$ This seems unnecessary and improbable In at least one of our patients significant amounts of labeled $T_4$ and $T_3$ were detected in the peripheral blood The intensely hyperplastic hyperfunctioning glands are thought to secrete hormone precursors as fast as they are formed leaving little in the gland for coupling into iodothyronines Theoretically the provision of unusually large amounts of iodide might enable these thyroids to produce normal amounts of hormone Observations on this point have not been made

*Genetics*

An unusual opportunity to study the genetics of this kind of goitrous cretinism has been beautifully exploited by McGirr and Hutchison [148]. Exhaustive studies of this closely knit and culturally isolated group in western Scotland have traced the complex family history through 160 years. The original male member came from Ireland and married his full cousin. There has been little marriage subsequently outside the tinker group but intermarriage within the group has been extremely frequent

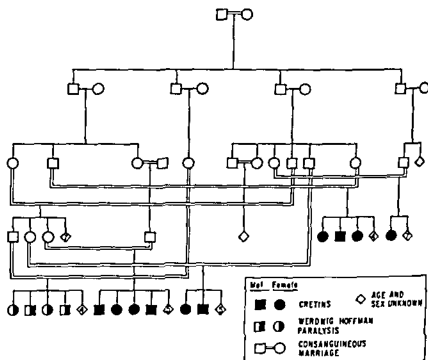



Fig 9-11 Pattern of heredity of cretinism with goiter as found by McGirr in a group of itinerant Scottish tinkers. Note particularly the remarkable number of consanguineous marriages. (By permission of The American Journal of Medicine.)

as can be seen in the genetic chart (Fig 9-11). Ten goitrous cretins are known to have appeared among 31 persons in four sets of siblings. In addition, there have been four cases of Werdnig Hoffman paralysis.

A study of the family tree shows that this form of cretinism with goiter behaves as a simple autosomal recessive gene. There is no sex predilection. The marriages which resulted in afflicted persons were all consanguineous but in no case was a parent afflicted. The inheritance ratio was somewhat in excess of the expected 1:3 in that there were 10 afflicted

siblings and 21 normal children in four sibships but Hutchison and McGirr point out that undoubtedly a number of unafflicted siblings were lost to genetic study [148] To date there have been no marriages of the afflicted members of this kindred so that there has been no opportunity to test inheritance from phenotypes

It seems a reasonable assumption that the cases of cretinism with goiter described from the Netherlands and from Scotland are homozygous The defect observed in these patients was quite complete There was no evidence whatsoever of any deiodination of DIT On the other hand five relatives of one of the Netherlands cases demonstrated defective DIT deiodinase activity and all had evidence of thyroid disease but without mental or skeletal retardation It follows that these relatives represent the heterozygous condition [149] It is provocative that one of the patients in this kindred had thyrotoxicosis and another had died with a diagnosis of thyrotoxicosis but a relationship between the deiodinase defect and thyrotoxicosis is not apparent

If as seems probable the gene which is defective in these patients is responsible for the production of iodotyrosine deiodinase then it might be supposed that in the heterozygous condition enough of the deiodinating enzyme would be produced to handle normal loads of the metabolite particularly if through thyroid hypertrophy enough hormone precursor could be retained in the thyroid to satisfy the demands of iodothyronine synthesis If this is the case it should be possible to test the deiodinase activity in heterozygotes by challenging the patient with an intravenous dose of labeled DIT to which an amount of carrier DIT was added which would overload the deiodinating capacity of the limited enzymatic endowment of the patient *There has been no opportunity to test this* hypothesis but if a normal individual is given between 25 and 50 mg labeled DIT then approximately 40 to 50 per cent of the labeled iodine appears in the urine as unchanged DIT If such a test could be performed on the goitrous but euthyroid relatives of a patient with full deiodinase defect cretinism and goiter a far higher fraction of the administered DIT might appear unchanged in the urine than in normal individuals If this hypothesis is proved true a method will be provided for detecting the carrier state of the deiodinase defect

## CRETINISM WITH IODINATED POLYPEPTIDES IN THE SERUM

The final category of patients with goiter and hypothyroidism which will be discussed in this chapter is less well defined and the biochemical mechanisms less well perceived than some of those which have already been discussed The patients are characterized by the appearance in the blood of iodinated amino acids which are in peptide linkage and which are not extractable into acid butanol as are the iodinated thyronines which normally appear in the peripheral blood

*Case Studies*

The first of these patients to come to the author's attention were two sisters in the clinic of McGirr at the Royal Infirmary in Glasgow [?]. Both were in their early twenties, were retarded mentally, were hypothyroid and had large nodular goiters. Each had been given a tracer of $I^{131}$, and samples of serum had been obtained serially for many hours. The peculiarity of the samples was that the labeled iodine of the serum was incompletely extractable into butanol. When the samples were acidified to pH 2 it was possible to extract only 50 to 60 per cent, whereas 85 to 95 per cent is readily extractable from normal serums. These studies were not pursued further at that time.

In 1956 Whitelaw, Thomas and Reilly reported in abstract an 8½ year old female cretin with a PBI concentration of 11.2 and 11.3 μg per 100 ml [129]. There was no goiter. The 24 hr $I^{131}$ uptake approached 100 per cent. Chromatographic analyses of samples of serum—performed in the laboratory of Werner, Block and Mandl [150]—disclosed a variety of iodinated compounds, but it was of particular interest that in three separate samples a large fraction of the labeled iodine was in the form of an unidentified iodoprotein.

In the following year DiGeorge and Pashkis described detailed studies on a 7½ year-old retarded female who had a high uptake and retention of $I^{131}$ [151]. There was a small goiter. The serum concentration of PBI was 7.7 and 9.6 μg per 100 ml. Chromatographic analyses were performed by J. Gross. In two successive samples of serum 85 and 63 per cent of the labeled iodine failed to extract into butanol. That which did extract was identified as $T_4$. The authors suggested that a calorigenically inefficient product was being secreted by the gland and that it was an iodoprotein. In an addendum they briefly reported another similar case.

A short while later Werner et al. [150] reported a 40-year-old female with signs and symptoms suggestive of hypothyroidism but without goiter. Protein bound iodine was 4.2 μg per 100 ml, and the 24-hr $I^{131}$ uptake was 25 per cent. Approximately one-third of the labeled iodine in the serum failed to extract into butanol. Upon hydrolysis in the presence of strong acid *MIT and DIT were reported in chromatograms of serum samples.*

Several patients with an abnormal iodinated component in their serums which is poorly soluble in butanol have been studied by DeGroot et al. in the author's laboratory [152, 153].

*Patient 1.* This patient was a 28-year-old cretinous female with short stature and imbecility. A large nodular goiter which was removed 10 years previously had shown hyperplasia on histologic examination. There was moderate regrowth of the thyroid when thyroid medication was discontinued several weeks prior to study in 1957.

The significant findings were a high uptake of $I^{131}$ and an iodinated fraction in the serum which was not extractable into butanol when the serum was acidified to pH 2. Only inorganic iodide was demonstrable in the urine. Deiodinization of administered labeled DIT was normal, and there was no discharge of labeled iodide from the gland after administration of KSCN. The half time of retention of labeled iodine in the gland was 18 days. The half time of retention of administered labeled $T_4$ was 8.5 days (normal).

Particular interest centered on the abnormal iodinated component in the serum. Extractability into butanol was increased by treatment of the serum

with proteolytic enzymes such as trypsin chymotrypsin or pepsin Chromatograms of these serum hydrolyzates revealed MIT and DIT in addition to iodide $T_4$ and $T_3$

The abnormal iodinated component of the serum was transported differently from $T_4$ When the serum was subjected to electrophoretic analysis in a starch supported barbital buffer at pH 8 6 the principal iodinated component was found as a single zone associated with the peak and the advanced limb of the albumin fraction (Fig 9 12) Although this component had an

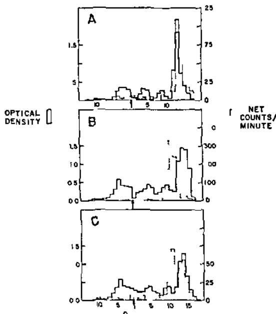



Fig 9-12 Starch block electrophoresis of serum of a patient with cretinism and goiter (see text) A Four days after administration of I B 12 hr after $I^{131}$ labeled $T_4$ administered intravenously C 4 days after a second dose of I The shaded area is labeled iodine The open areas correspond to protein Note the presence of a labeled compound corresponding to albumin in A and C and in B a failure of T to conform to albumin or the in vivo labeled iodine (*By permission from The Journal of Clinical Endocrinology and Metabolism*)

electrophoretic mobility of normal human serum albumin it could not be precipitated with an antiserum against normal serum albumin produced in the horse

Thyroxine was found in its usual position in the interalpha zone where it was presumably in association with the normal T binding protein (Fig 9 12) When $I^{131}$ labeled $T_4$ was added to the serum of this patient or to the serum of normal patients it proved to be more than 90 per cent extractable under conditions which gave only a 60 or 65 per cent extractability of the iodinated components of the patient s serum labeled in vivo Thus there was no abnormality of the thyroid binding protein of the serum

*Patient 2* A 10-year-old female with retarded mental and physical development and a goiter was studied 2 months after discontinuance of medi

cation with desiccated thyroid An $I^{131}$ uptake was 66 per cent in 2 hr and 71 per cent in 24 hr The half time of the labeled iodine in the gland was 12 days There was no release of labeled iodine from the gland by KSCN Approximately half the labeled iodine of the acidified serum was insoluble in butanol Sixty three per cent traveled with albumin on starch block electrophoresis The butanol solubility of the labeled iodine of the serum was increased to 91 per cent following enzymatic digestion Both iodide and $T_4$ were present in chromatograms of butanol extracts of the serum and labeled components with the mobility of MIT and DIT and of iodothyronines were present in chromatograms of samples of urine Twenty per cent of the labeled iodine in the urine was not soluble in butanol

*Patient 3* A 42 year-old mentally dull male was known to have had a goiter since age 14 It was removed at age 23 but subsequently recurred A mother and sister had goiter There was a 60 per cent uptake of labeled iodine at 2 hr and 82 per cent in 24 hr The half time of the labeled iodine in the gland was 145 days The concentration of the labeled iodine in the serum was only 0 05 per cent per liter at 48 hr Of this 24 per cent was not extractable into butanol The extractability was increased to 90 per cent by digestion with chymotrypsin Chromatograms of the serum revealed only iodide and $T_3$ and $T_4$ Chromatography of the urine disclosed only iodide

Detailed studies were performed on thyroid tissue received at the time of subtotal thyroidectomy Deiodinization of MIT by gland homogenate was normal Chromatographic analysis for the iodinated amino acids after enzymatic digestion disclosed a normal distribution except for a somewhat reduced amount of $T_3$ and $T_4$ Electrophoretic analysis of a saline extract of this gland showed two major components one of which occupied the electrophoretic position of normal thyroglobulin The other had the mobility of serum albumin and contained no $I^{131}$ Preparative electrophoresis on a paper curtain failed to resolve this prethyroglobulin component Ultracentrifugal analysis of the $I^{131}$-containing peak from the preparative electrophoresis revealed material which had a low sedimentation constant There was not only a zone of normal thyroglobulin with an $S_{20,w} = 19$ but a much larger component with an $S_{20,w} = 4$ The $I^{131}$ protein in the thyroid saline extract was salted out of solution by 2 to 2 3 *M* phosphate buffer

*Patient 4* A 14 year-old schoolgirl with normal development was found to have had a goiter at age 4 The thyroid was four times enlarged The PBI concentration of the serum was 3 μg per 100 ml $I^{131}$ uptake was 50 per cent in 1 hr and 85 per cent in 24 hr The half time of retention in the gland was 8 3 days The serum $I^{131}$ concentration at 48 hr was 0 44 per cent per liter and at that time 30 per cent of the $PBI^{131}$ was not extractable into butanol Chymotrypsin digestion increased the extractability to 93 to 95 per cent Fifteen per cent of the labeled iodine in the urine was not extracted by butanol but all was dialyzable The iodinated component of the urine was not identified but in a butanol–acetic acid solvent system it remained close to the origin

A number of studies were done on a biopsy specimen of the thyroid A whole homogenate deiodinated MIT and DIT No thyroglobulin was found On electrophoretograms the labeled iodine was found in a zone close to the origin in the region which in serum is occupied by the β gamma globulins Ultracentrifugal analysis showed only one peak at $S_{20,w} = 4$ Salting out with phosphate buffer was abnormal There was no sharp separation with increasing salt concentration but rather a gradual salting out between 2 and 2 5 *M*

*Patients 5 and 6* Samples of serum and urine were received from B Courvoisier of Geneva, Switzerland They were obtained from two male siblings age 15 and 17 who had been hypothyroid and goitrous during most of their lives The PBI of the first of these was 5 2 and the $I^{131}$ uptake at 24 hr was 90 per cent The serum at 72 hr contained 0 69 per cent of the dose per liter Approximately 60 per cent of the $PBI^{131}$ was not extractable into butanol Thyroxine $T_4$ and iodide were identified in the serum and possibly small amounts of MIT and DIT The butanol extractability of the serum $I^{131}$ was increased by chymotrypsin digestion The butanol insoluble component traveled with albumin on paper electrophoresis It did not salt out with phosphate buffer at concentrations below 2 2 M Substances were found in the serums which had the $R_F$ of iodide MIT and DIT in the butanol–acetic acid solvent system There was also an unidentified component with an $R_F$ less than iodide

The PBI of the brother was 1 5 µg per 100 ml and the $I^{131}$ uptake was 84 per cent At 96 hr the serum contained only 0 1 per cent of the dose per liter Only iodide was identified with certainty in the serum but the concentration of labeled iodine was too low for satisfactory studies Thirty five per cent of the $PBI^{131}$ was not extractable into butanol A nonbutanol extractable component was present in a concentration of 15 per cent in the urine collected on the fourth day

A retarded patient the product of a first-cousin marriage has recently been reported in detail [*168a*] Observations very similar to those on Patient 1 above were obtained The labeled iodine in the serum was poorly extractable into butanol and on electrophoresis moved with albumin

*Physiological Interpretation*

The differences observed among the patients of this category may possibly be attributable to variations existing among patients who have in common certain fundamental abnormal characteristics All showed evidence of hypothyroidism and all had goiter which appeared at an early age All had in addition an iodinated component in the peripheral blood which was identified by labeling with $I^{131}$ and which was not extractable from the acidified serum into *n* butanol Three had an unidentified iodinated component in the urine which was not extractable into butanol and which was dialyzable The other two did not have this finding One of the patients whose thyroid tissue was available had no thyroglobulin and the other had an increased amount of a component with an $S_{20,w} = 4$ Two of the patients had iodinated components in the peripheral blood which had the chromatographic characteristics in the butanol–acetic acid solvent system of MIT and DIT

The nature of the circulating iodinated component in the peripheral blood was of particular interest Upon hydrolysis with trypsin or chymotrypsin iodinated MIT DIT $T_3$ and $T_4$ were released and the butanol solubility of the labeled iodine was increased

The data presently available do not permit an exact interpretation of the abnormality The thyroid is secreting an abnormal iodinated component into the blood which is calorigenically ineffective This com

ponent is hydrolyzable with crude trypsin chymotrypsin, and pepsin to release iodotyrosines and iodothyronines

The significant question which cannot be answered at present regards the reason for the production and secretion of an abnormal polypeptide Such production could conceivably be due to an abnormality in the synthesis of thyroglobulin (i e the formation of an abnormal thyroglobulin in a way analogous to genetically controlled synthesis of abnormal hemoglobins) or to defective proteolytic degradation of thyroglobulin because of either an altered nature of the thyroglobulin itself, or a defect in one of the proteolytic enzymes of thyroglobulin degradation At present it is impossible to exclude any of these possibilities and indeed all may be operative in different groups of patients Before a final answer can be given it will be necessary to search for abnormal thyroglobulins and for abnormalities in the enzymatic components of the stepwise scheme of thyroglobulin proteolysis

*Genetics*

Too few patients have been studied for the genetics of this disease or group of diseases to be characterized The two sisters in Dr McGirr's clinic and the two brothers in the clinic of Dr Courvoisier strongly suggest that the disease is heredofamilial In so far as is known no affected parents have been found in the kindred of any one of these patients except that the mother of one had an asymptomatic goiter

## DIAGNOSIS

There are no distinguishing clinical features among the various types of familial goiter with hypothyroidism except for that group which has an associated nerve deafness Differential diagnosis of the various categories which have been described must in general depend upon specific laboratory tests [103] There is sufficient information already available on many patients described in the literature on which to base a retrospective presumptive diagnosis as has been seen This is not possible for several interesting case reports which have not been mentioned because of insufficient data [155-159]

The most useful preliminary observation is the uptake and retention of labeled iodine by the thyroid gland Characteristically these patients have an unusually rapid uptake curve which reaches high levels within the first hour or two following administration Patients with the organification defect lose the labeled iodine from the gland rapidly after the peak uptake value is reached and this rate of loss may be made precipitous by the administration of perchlorate or thiocyanate The same is true of those patients with associated congenital goiter and nerve deafness Diagnosis is made certain if it can be shown that there is no organically bound iodine in the removed gland

Patients who have the iodotyrosine coupling defect also have a high rate of uptake of the labeled iodine but it is retained in the gland and disappears with only a moderate acceleration of the normal half time The diagnosis in these patients can be made with reasonable certainty only by showing that a specimen of thyroid tissue removed 72 hr or later following administration of labeled iodine contains an abundance of labeled MIT and DIT but no detectable labeled $T_4$ or hardly any No abnormal iodinated substances should be present in the blood of such a patient

Patients with the deiodinase defect also have a rapid uptake phase following administration of labeled iodine There is rapid loss of labeled iodine from the gland after the peak uptake value is reached followed by a slower loss beginning 24 hr or so after administration A few patients who undoubtedly belong to this group do not have this phase of rapid release Chromatographic analysis of blood and urine discloses the presence of iodinated substances which have the chromatographic mobility of MIT and DIT and their derivatives and conjugates The final test is to show that an administered dose of labeled DIT is excreted almost or entirely unchanged in the urine

Patients with congenital goiter and hypothyroidism who have butanol insoluble iodinated components in their serums have normal or elevated retention curves of labeled iodine Diagnosis depends upon demonstration in the acidified serum of butanol unextractable iodine and the further demonstration that proteolytic hydrolysis of this component yields the usual iodinated thyronines and tyrosines It seems entirely possible that this group is heterogeneous and that with further study it will be subdivided into several groups according to specific abnormalities of the synthetic and degradative schemes for thyroglobulin in the thyroid gland

The discussion thus far has centered on the diagnosis in patients who have a complete defect i e homozygous expre ion An important problem and a more difficult one from many points of view is the diag no is of specific genetic defect in patients who may be heterozygous for that defect From studies done to date it is clear only in the deiodinase defect group that the heterozygous state may cause disease In one case it was possible to show that various goitrous members of the family of a patient with fully expre sed cretinism and deiodina e defect were less able to deiodinate injected DIT than normal persons More recent studies have shown that increasing the dose of DIT to approximately 25 mg significantly loads the deiodinating system in vivo in normal man [160] It is quite possible that this load might completely overwhelm the deiodinating system in a ubject with the deiodinase defect in the heterozygous state

There is now evidence that certain patients with nodular goiter but without evidence of pre ent or past hypothyroidism may have small but

detectable amounts of nonbutanol-extractable iodine in their serums [163] It remains to be shown that these patients are heterozygous in relation to patients homozygous for the same defect who have congenital goiter and hypothyroidism *Unfortunately in these patients the* glands are so filled with iodine and so sluggish in their activity that the turnover of labeled iodine is extremely low Manipulations with the small amounts of labeled iodine appearing in the serum after tracer doses are difficult to the point of uncertainty

## TREATMENT

Satisfactory treatment of familial goiter depends upon the stage of development of the local disease and the degree to which irreversible changes have occurred in the skeleton and central nervous system Remarkable shrinkage of the goiter may be expected from treatment with L-thyroxine or desiccated thyroid in usual maintenance doses providing irreversible changes of degeneration cyst formation and fibrous replacement have not taken place The goiter will inevitably recur if medication is discontinued

Care should be exercised in treating a familial goiter in view of the tendency of some of them to undergo malignant change In general it would be wise to remove any nodule which fails to shrink after several weeks of replacement therapy Unfortunately this will often be the case with well-established goiters so that more often than not these patients eventually require surgery Desiccated thyroid is required after thyroidectomy unless it is desirable to maintain the patient in a myxedematous state

Unless treatment is begun early within the first few weeks of life there is considerable risk of permanent retardation of intellectual development or skeletal growth There is reason to suspect that damage may occur in utero so that no amount of replacement therapy will prevent developmental arrest and permanent retardation

Medication should be sufficient Perhaps a safe rule is to give increasing doses of desiccated thyroid until the first signs of overdosage appear (tachycardia hyperactivity) and then reduce the dosage slightly Particular attention should be paid to newborn siblings of patients who have familial goiter Carr et al [161] have recently reported a mother who produced an apparently normal infant after two successive cretins by taking large doses of desiccated thyroid during pregnancy The child was later shown to have no thyroid gland Presumably the large doses caused sufficient placental transfer of hormone to permit normal development of the fetus

Management of the patient with fully developed goiter hypothyroidism and permanent retardation is unsatisfactory Little is accomplished in the adult by replacement therapy and as often as not inac-

ceptable aggressiveness or other undesirable behavior may be the result of full thyroid medication In these patients the dosage is best adjusted to that which keeps the patient active and comfortable without arousing unwanted side effects

A few patients with familial goiter such as some of those who have associated eighth nerve deafness appear to manufacture adequate amounts of hormone and to develop normally They require thyroid substance only to prevent growth of the small goiters to which they are predisposed

## SUMMARY

1 The synthesis storage secretion delivery and utilization of the thyroid hormones involve a complex sequence of metabolic events each of which is probably dependent upon specific enzymatic activity Thyroid disease may result from blockage at many steps in this metabolic process

2 Familial goiter and hypothyroidism may occur when there is failure to convert inorganic iodide into iodine in the thyroid gland Accumulated iodide is precipitously discharged from the gland upon the administration of thiocyanate

3 A group of patients possibly closely related to the above have goiter and congenital nerve deafness Labeled iodine in the gland is partially discharged upon the administration of thiocyanate

4 Three families of patients have now been discerned who have a defect in the coupling of iodotyrosines into iodothyronines The disease can be demonstrated with certainty only when it is shown that iodothyronines fail to appear in biopsy specimens of thyroid tissue

5 Several family groups are known to be unable to deiodinate iodotyrosines Loss of hormone precursors from the gland and into the urine accounts for hypothyroidism and compensatory goiter Several goitrous but otherwise normal relatives of one such patient have been shown to deiodinate diiodotyrosine less well than normal individuals The condition is most convincingly diagnosed by the demonstration that intravenously administered labeled DIT is excreted intact in the urine

6 Certain patients with congenital goiter have been found with a circulating butanol insoluble iodinated component in the serum Certain slight differences among patients suggest that this group may be heterogeneous The disease may represent a defect in thyroglobulin synthesis or proteolytic degradation in the thyroid gland

## BIBLIOGRAPHY

1 Wilkins L Clayton G W and Berthrong M Development of goiters in cretins without iodine deficiency hypothyroidism due to apparent inability of the thyroid gland to synthesize hormone Pediatrics 13 235 1954

2 Stanbury J B and McGirr E M Sporadic or nonendemic familial cretinism with goiter Am J Med 22 71 1957
3 Clayton C W Hypothyroidism and goiter due to defects in the intrathyroidal synthesis of thyroxine Am J M Sc 236 7 0 1958
4 Stanbury J B and Querido A Genetic and environmental factors in cretinism a classification J Clin Endocrinol 16 1522 1956
5 Lelong M Joseph R Canlorbe P Job J C and Plainfosse B Hypothyroidie par anomalie congénitale de l hormonogénèse (cinq observations) Arch franç pédiat 13 1 1956
6 Joseph R Tubiana M and Job J C Hypothyroidie par anomalie congénitale de la thyroxinogenèse Rev franç étud clin et biol 3 167 1958
7 Osler W Sporadic cretinism in America Tr Cong Am Phys & Surg 4 169 1897
8 Berson S A Pathways of iodine metabolism Am J Med 20 653 1956
9 Riggs D S Quantitative aspects of iodine metabolism in man Pharmacol Rev 4 281 1952
10 Barker S B Thyroid Ann Rev Physiol 17 417 1955
11 Wolff J and Goldberg R C Disorders of iodine metabolism in *Biochemical Disorders in Human Disease* edited by R H S Thompson and E J King p 289 Academic Press Inc New York 1957
12 Roche J and Michel R Nature biosynthesis and metabolism of thyroid hormones Physiol Rev 35 583 1955
13 Riggs D S and Stanbury J B Unpublished observations
14 Slingerland D W The influence of various factors on the uptake of iodine by the thyroid J Clin Endocrinol 15 131 1955
15 Freinkel N and Ingbar S H Effect of metabolic inhibitors upon iodide transport in sheep thyroid slices J Clin Endocrinol 15 598 1955
16 Halmi N S Regulation of the rat thyroid in short term iodine deficiency Endocrinology 54 216 1954
17 Vanderlaan W P and Caplan R Observations on a relationship between total thyroid iodine content and the iodide-concentrating mechanism of the thyroid gland of the rat Endocrinology 54 437 1954
18 Wyngaarden J B Stanbury J B and DuToit C H On the mechanism of iodide accumulation by the thyroid gland J Clin Endocrinol 11 1259 1951
19 Wyngaarden J B and Stanbury J B The formation of monoiodotyrosine and an intermediate iodine complex by thyroid homogenates J Biol Chem 212 151 1955
20 VanderLaan W P and Bissell A Effects of propylthiouracil and of potassium thiocyanate on the uptake of iodine by the thyroid gland of the rat Endocrinology 39 157 1946
21 Wyngaarden J B Wright B M and Ways P The effect of certain anions upon the accumulation and retention of iodide by the thyroid gland Endocrinology 50 537 1952
22 Wood J L and Kingsland N Labeled sulfur uptake by thyroids of rats with low plasma thiocyanate levels J Biol Chem 185 833 1950
23 Maloof F and Soodak M The inhibition of the metabolism of thiocyanate in the rat thyroid Endocrinology 69 106 1959
24 Taurog A Potter G D and Chaikoff I L Conversion of inorganic I to organic I[131] by cell free preparations of thyroid tissue J Biol Chem 213 119 1955
25 Mayer S W Dimick B W and Kelly F H Further observations on the I[131] metabolism of prefrozen thyroid slices Endocrinology 59 586 1956
26 Schachner H Franklin A L and Chaikoff I L The effect of cytochrome oxidase inhibitors on the formation in vitro of thyroxine and diiodotyrosine by thyroid tissue with radioactive iodine as indicator J Biol Chem 151 191 1943
27 Deltolertis P and Carro R Peroxidase activity of the thyroid gland under normal and experimental conditions Endocrinology 38 137 1946
28 Kracht J and Kracht U Zum histochemischen Peroxydasenachweis in der

Schilddruse unter normalen und pathologi chen B dingungen Arch exper I ath u l harmakol 213 4 J 1J51
J D mpsey L W Fluorescent and hi tochemical reactions in the rat thyroid gland at different states of physiologi al activity I ndocrinology 34 27 1944
30 Serif G S and Kirkwood S Enzyme systems concerned with the synthesis of monoiodotyrosine J Biol Chem 233 10J 1958
31 Rosenberg I N The antithyroid activity of some compounds that inhibit peroxidase Science 116 503 1952
32 Randall L O Reaction of thiol compounds with peroxida e and hydrogen peroxide J Biol Chem 164 5 1 1946
33 Alexander N M Iodide peroxidase in rat thyroid and salivary glands and its inhibition by antithyroid compounds Fed I roc 18 179 1959
34 Fawcett D M and Kirkwood S The synthesis of organically bound iodine by cell free preparations of thyroid tissue J Biol Chem 205 795 1953
35 Weiss B Utilization of radioactive iodine by cell free preparations of beef thyroid tissue J Biol Chem 201 31 1953
36 Lamberg B A Matovinovic J and Stanbury J B The in vitro formation of iodinated compounds by thyroid sl ces and homogenate preparations Acta endocrinol 29 33 1958
37 Taurog A Tong W and Chaikoff I L An unidentified iodine compound formed by incubation of cell free preparations of tis ue with iodide I J Biol Chem 227 759 1957
38 Tong W Taurog A and Chaikoff I L Activation of the iodinating system in sheep thyroid particulate fractions by flavin cofactors J Biol Chem 227 773 1957
39 Pitt Rivers R Galton V A and Halmi N S Nature of radioiodine not dischargeable with perchlorate in the thyroid glands of th ouracil treated rats Endocrinology 63 6JJ 1958
40 Harington C Twenty five years of re earch on the biochemistry of the thyroi l gland Endocrinology 49 401 1951
41 von Mutzenbecher I Uber die Bil lung v n Thyroxin au Dijo ltyro in Zts f r physiol Chem 261 253 1939
42 Harington C and Pitt Rivers R The chemical conversion of di odotyrosine into thyroxine B ochem J 39 157 1945
43 I itt Rivers R The oxidation of diiodotyro ine derivatives B hem J 43 2 3 1948
44 I itt Rivers R The action of antithyro d substances on the f rmation in vit o of acetylthyroxine from acetyl-diiodotyrosine Biochim et I iophys acta 2 311 1948
45 Roche J Michel R and Etling N Sur la b osynthèse des hormone thyroid iennes in vit o Compt rend soc b ol 148 1159 1J54
46 Roche J Michel R Wolf W and Nunez J Sur la présence lar s la thyro globul ne de la 3 3 -diiodothyronine nouvelle hormone th yroïdienne Con pt rend acad sc 240 921 1955
47 Roche J Michel R and Nuñez J Sur la présence de la 3 3 5 triiodothy ron ne dans le sang de rat Compt rend soc biol 150 0 1956
48 Stanbury J B and Morris M L The metabolism of 3 3 di odothyronine in man J Clin Endocrinol 17 13 4 1957
49 Dunn J and Stanbury J B The metabolism of 3 3 5 tri odothyronine in m n J Clin Endocrinol 18 13 1958
50 Robbins J Wolff J and Rall J E Iodoproteins in thyroid ti ue an l blood of rats with a transplantable thyroid tumor Endocrinology 64 1 1959
51 Robbins J Wolff J and Rall J E Iodoproteins in norm al and abnormal human thyro d tissue and in normal sheep thyroid En lo rinolo gy 64 37 19 9
52 Ingbar S H Askonas B A and Work T S Ob ervations concerning the heterogeneity of ovine thyroglobul n Endocrinology 64 110 1959
53 Stanbury J B Unpubl shed ob ervation
54 Roche J Mi h l R Nunez J and Lacombe G Sur la présence d acides

aminés iodés N terminaux dans la thyroglobuline de porc Compt rend acad sc 240 404 1955
55 Tong W and Chaikoff I L Hydrolysis of I[131] thyroprotein by pancreatic enzymes J Biol Chem 232 939 1958
56 Stanbury J B Brownell C L Riggs D S Perinetti H Itoiz J and del Castillo E B Endemic Goiter The Adaptation of Man to Iodine Deficiency Harvard University Press Cambridge 1954
57 Laver W G and Trikojus V M Purification of thyroid protease by acetone fractionation Biochim et biophys acta 16, 592 1955
58 Laver W C and Trikojus V M Complex nature of the proteolytic system of the thyroid gland Biochim et biophys acta 20 444 1956
59 Weiss B Peptidase and proteinase activity of beef thyroid tissue J Biol Chem 205 193 1953
60 Alpers J B Robbins J and Rall J E The hydrolysis of rat thyroglobulin by thyroidal enzymes Endocrinology 56 110 1955
61 DeRobertis E Cytological and cytochemical bases of thyroid function Ann New York Acad Sc 50 317 1949
62 Hartmann N Über den Abbau von Dijodtyrosin im Gewebe Ztschr physiol Chem 285 1 1950
63 Roche J Michel R, Michel O and Lissitzky S Sur la déshalogenation enzymatique des iodotyrosines par le corps thyroïde et sur son rôle physiologique Biochim et biophys acta 9, 161 1952
64 Roche J Michel R Gorbman A and Lissitzky S Sur la déshalogenation enzymatique des iodotyrosines par le corps thyroïde et sur son rôle physiologique II Biochim et biophys acta 12 570 1953
65 Stanbury J B The requirement of monoiodotyrosine deiodinase for triphosphopyridine nucleotide J Biol Chem 228 801 1957
66 Stanbury J B and Morris M L Deiodination of diiodotyrosine by cell free systems J Biol Chem 233 106 1958
66a Carr E A Beierwaltes W H Spafford N R Duncan L L and Stambaugh R A Iodotyrosine deshalogenase in diseased human thyroids J Clin Endocrinol 19 1582 1959
67 Ingbar S H and Freinkel N Regulation of the peripheral metabolism of the thyroid hormones Recent Prog Hormone Res 16 1960
68 Horst W and Rosler H Der Transport des Hormonjods im menschlichen Serum untersucht mit Papierelektrophorese und Radiojod Zugleich ein Beitrag zur Frage der Existenz von Sog Zwischenfraktionen Klin Wehnschr 31 13 1953
69 Robbins J and Rall J E The interaction of thyroid hormones and protein in biological fluids Recent Prog Hormone Res 13 161 1957
70 Rich C and Bearn A G Localization of the thyroxine-binding protein of serum by starch gel electrophoresis Endocrinology 62 68 1958
71 Ingbar S H Pre-albumin A thyroxine-binding protein of human plasma Endocrinology 63 256 1958
72 Deiss W P Albright E C and Larson F C Comparison of in vitro serum protein binding of thyroxin and triiodothyronine Proc Soc Exper Biol & Med 84 513 1953
73 Roche J Michel R and Tata J Sur l'excrétion biliaire et la glycuroconjugaison de la 3 5 3' triiodothyronine Biochim et biophys acta 11 543 1953
74 Isselbacher K J Enzymatic mechanisms of hormone metabolism II Mechanism of hormonal glucuronide formation Recent Prog Hormone Res 12 134 1956
75 Tata J R Rall J E and Rawson R W Metabolism of L-thyroxine and L-3 5 3' triiodothyronine by brain tissue preparations Endocrinology 60 83 1957
76 Albright E C Larson F C and Tust R H In vitro conversion of thyroxin to triiodothyronine by kidney slices Proc Soc Exper Biol & Med 86 137 1954
77 Larson F C Tomita K and Albright E C The deiodination of thyroxine to

triiodothyronine by kidney slices of rats with varying thyroid function Endocrinology 57 338 1955
78 Albright E C Tomita K and Larson F C *In vitro* metabolism of triiodothyronine Endocrinology 64 208 1959
79 Sprott W E and Maclagan N F Metabolism of thyroid hormones deiodination of thyroxine and triiodothyronine *in vitro* Biochem J 59 288 1955
80 Maclagan N F and Reid D The deiodination of thyroid hormones *in vitro* Ciba Foundation Colloquia on Endocrinology 10 190 1957
81 Cruchaud S Vannotti A Mahaim C and Deckelmann J The *in vitro* effect of methylthiouracil and oestradiol monophosphate on the conversion of thyroxine to triiodothyronine by kidney slices Lancet 2 900 1955
82 Yamazaki E and Slingerland D W The *in vitro* metabolism of thyroxine triiodothyronine and their acetic and propionic acid analogues Endocrinology 64 126 1959
83 Stanbury J B Unpublished observations
84 Selenkow H A and Asper S P Biological activity of compounds structurally related to thyroxine Physiol Rev 35 4 6 1955
85 Money W L Meltzer R I Young J and Rawson R W The effect of change in chemical structure of some thyroxine analogues on the metamorphosis of *Rana pipiens* tadpoles Endocrinology 63 20 1958
86 Mussett M V and Pitt Rivers R The thyroid like activity of triiodothyronine analogues Lancet 2 1212 1954
87 Barker S B Mechanism of action of thyroid hormone Physiol Rev 31 205 1951
87a Nikkila E A and Pitkänen E Liver enzyme pattern in thyrotoxicosis Acta endocrinol 31 573 1959
88 Wolff J and Wolff L C The effect of thyroxine on isolated dehydrogenases Biochem et biophys acta 26 387 1957
89 Wolff E C and Ball E G The action of thyroxine on oxidation of succinate and malate J Biol Chem 224 1083 1957
90 Barker S B *In vitro* action of thyroxine analogs on succinate and malate oxidation Endocrinology 61 534 1957
91 Tapley D F Cooper C and Lehninger A L The action of thyroxine on mitochondria and oxidative phosphorylation Biochim et biophys acta 18 597 1955
92 Emmelot P and Bos C J Thyroxine mediated release of diphosphopyridine nucleotide from mitochondrial dehydrogenases Exper Cell Res 14 13 1958
93 Gershoff S N Vitale J J Antonowicz I Nakamura M and Hellerstein E E Studies of interrelationships of thyroxine magnesium and vitamin B J Biol Chem 231 849 1958
94 Peterson R L The influence of the thyroid on adrenal cortical function J Clin Invest 37 736 1958
95 Yates F L Urquhart J and Herbst A L Effects of thyroid hormones on ring A reduction of cortisone by liver Am J Physiol 195 373 1958
96 McGuire J and Tomkins G Effect of thyroxin administration on the rate and steric course of enzymatic reduction of steroids Nature London 182 261 1958
97 Lardy H A and Maley G F Metabolic effects of thyroid hormones *in vitro* Recent Progr Hormone Res 10 129 1954
98 Martius G and Hess B The mode of action of thyroxin Arch Biochem 33 486 1951
99 Maley G F and Lardy H A Efficiency of phosphorylation in selected oxidations by mitochondria from normal and thyrotoxic rat livers J Biol Chem 215 377 1955
100 Hoch F L and Lipmann F The uncoupling of respiration and phosphorylation by thyroid hormones Proc Nat Acad Sc 40 909 1954
101 Taurog A Tong W and Chaikoff I L Thyroid I metabolism in the absence of the pituitary the untreated hypophysectomized rat Endocrinology 62 646 1958

102 Rawson R W Physiological reactions of the thyroid-stimulating hormone Ann New York Acad Sc 50 491 1949
103 Umbarger H E and Brown B Isoleucine and valine metabolism in *Escherichia coli* J Biol Chem 233 415 1958
104 Collberg R C Wolff J and Greep R O Studies on the nature of the thyroid pituitary interrelationship Endocrinology 60 39 1957
105 Greer M A and Erwin H The location of the hypothalamic area controlling *the secretion of thyrotropin by the pituitary J Clin Invest 33 938 1954* (abstract)
106 Harris G W *Neural Control of the Pituitary* E Arnold London 1955
107 Bogdanove E M and D'Angelo S A The effects of hypothalamic lesions on goitrogenesis and pituitary TSH secretion in the propylthiouracil treated guinea pig Endocrinology 64 53 1959
108 Uotila U U The role of the pituitary stalk in the regulation of the anterior pituitary with special reference to the thyrotropic hormone Endocrinology 25 *605 1939*
109 Cortell R and Rawson R W The effect of thyroxin on the response of the thyroid gland to thyrotropic hormone Endocrinology 35 488 1944
110 Stanbury J B and Hedge A N A study of a family of goitrous cretins J Clin Endocrinol 10 1471 1950
111 Stanbury J B Cretinism with goiter a case report J Clin Endocrinol 11 740 1951
112 Stanbury J B Ohela K and Pitt Rivers R The metabolism of iodine in 2 goitrous cretins compared with that in 2 patients receiving *methimazole J* Clin Endocrinol 15 54 1955
113 Schultz A Fink L B Kennedy B J and Zieve L Exchangeable character of accumulated I[131] in the thyroid gland of a goitrous cretin J Clin Endocrinol 17 441 1957
114 Clayton C W Smith J D and Leiser A Familial goiter with defect in intrinsic metabolism of thyroxine without hypothyroidism J Pediat 52 129 1958
✓ 114a *Haddad H M and Sidbury J B Jr Defect of the iodinating system in* congenital goitrous cretinism report of a case with biochemical studies J Clin Endocrinol 19 1416 1959
✓ 115 Gardner J A Hayles A B Woolner L B and Owen C A Iodine metabolism in goitrous cretins J Clin Endocrinol 19 638 1959
✓ 116 Federman D Robbins J and Rall J E Some observations on cretinism and its treatment New England J Med 259 610 1958
✓ 117 Jackson A D M Non-endemic goitrous cretinism Arch Dis Childhood *29 571 1954*
118 Brain W R Heredity in simple goitre Quart J Med 20 303 1927
119 Johnsen S Familial deafness and goitre in persons with a low level of protein bound iodine Acta oto laryng Suppl 140 168 1958
120 Elman D S Familial association of nerve deafness with nodular goiter and thyroid carcinoma New England J Med 259 219 1958
✓ 120a McGirr E M Hutchison J H and Clement W E Sporadic goiter due to dyshormonogenesis impaired utilization of trapped iodide Scot M J 4 107 *1959*
121 Roberts K D Thyroid carcinoma in childhood in Great Britain Arch Dis *Childhood 32 58 1957*
122 Thieme L T A report of the occurrence of deafmutism and goiter in four of six siblings of a North American family Ann Surg 146 941 1957
✓ 123 Morgans M E and Trotter W R Association of congenital deafness with goitre Lancet 1 607 1958
123a Buchanan J and Crooks J Goitrous cretinism Scot M J 2 115 1959
124 Deraemaeker R Congenital deafness and goiter Am J Human Genet 8 253 1956
125 Gross J and Pitt Rivers R 3 5 3 triiodothyronine I Isolation from thyroid gland and synthesis Biochem J 53 645 1953

126 Mosier H D Blizzard R M and Wilkins L Congenital defects in the biosynthesis of thyroid hormone Report of two cases Pediatrics 21 248 1958

127 Joseph R and Job J C L hypothyroidie congénital avec anomalie de la condensation des iodotyrosines Arch franç pédiat 15 1 1958

128 Werner S C Block R J Mandl R H and Kassenaar A A H Pathogenesis of a case of congenital goiter with abnormally high levels of SPI and with mono- and diiodotyrosine in the serum J Clin Endocrinol 17 817 1957

129 Whitelaw M J Thoms S and Reilly W A A nongoitrous cretin with a high level of serum PBI and thyroidal I131 uptake J Clin Endocrinol 16 983 1956 (abstract)

130 Lerman J Jones H W and Calkins E Studies on two sporadic cretinous brothers with goiter together with some remarks on the relation of hyperplasia to neoplasia Ann Int Med 25 677 1946

131 Hamilton J G Soley M H Reilly W A and Eichorn K B Radioactive iodine studies in childhood hypothyroidism Am J Dis Child 66 495 1943

132 Pickering D E Sheline G E and Crane J T Sporadic familial goitrous hypothyroidism A M A Am J Dis Child 93 510 1957

133 Zondek H Leszynsky H E and Zondek G W Triiodothyronine in myxedema and familial sporadic cretinism with goiter Acta endrocrinol 18 117 1955

134 Hubble D Familial cretinism Lancet 1 1112 1953

135 McGirr E M and Hutchison J H Radioactive iodine studies in nonendemic goitrous cretins Lancet 1 1117 1953

136 Hutchison J H and McGirr E M Hypothyroidism as an inborn error of metabolism J Clin Endocrinol 14 869 1954

137 Stanbury J B Kassenaar A A H Meijer J W A and Terpstra J The occurrence of mono- and diiodotyrosine in the blood of a patient with congenital goiter J Clin Endocrinol 15 1216 1955

138 Stanbury J B Meijer J W A and Kassenaar A A H The metabolism of iodotyrosines II The metabolism of mono and diiodotyrosine in certain patients with familial goiter J Clin Endocrinol 16 848 1956

139 Stanbury J B Kassenaar A A H and Meijer J W A The metabolism of iodotyrosines I The fate of mono- and diiodotyrosine in normal subjects and in patients with various diseases J Clin Endocrinol 16 735 1956

140 Albert A and Keating F R Jr Metabolic studies with I131 labeled thyroid compounds distribution and excretion of radiodiiodotyrosine in human beings J Clin Endocrinol 11 996 1951

141 Querido A Stanbury J B Kassenaar A A H and Meijer J W A The metabolism of iodotyrosines III Diiodotyrosine deshalogenating activity of human thyroid tissue J Clin Endocrinol 16 1036 1956

142 McGirr E M and Hutchison J H Sporadic cretinism with goitre Lancet 1 106 1956

143 McGirr E M Hutchison J H and Clement W E Sporadic non-endemic goitrous cretinism Identification and significance of monoiodotyrosine and diiodotyrosine in serum and urine Lancet 2 906 1956

144 Horst W Radiojoddiagnostik von Struma und Schilddrusenkrebs und Untersuchung zur Frage einer Jodfehlverwertung in deren Pathogenese Verh d 4 Jahrestagung des Deutschen Zentralausschusses für Krebsbekampfung und Krebsforschung Edited by A Dietrich Sonderbd zur Strahlentherap 34 150 1956

145 Costa A Cottino F Ferraris G M Marchis I Marocco F Mortara M and Pietra R Richerche sulla patogenesi del cretinismo endemico Med ina (Parma) 3 4 1953

146 Bernheim M and Berger M Le diagnostic et le mécanisme de l insuffisance thyroïdienne de l enfant étudiés à la faveur des épreuves à l radio iode Semaine hôp Paris 30 3575 1954

147 Burrell C D and Gardener D M T Cretinism in three siblings Proc Roy Soc Med 48 10 1955

148 Hutchison J H and McGirr E M Sporadic nonendemic goitrous cretinism Lancet 1 1035 1956
149 Metabolism of iodotyrosines (leading article) Lancet 2 611 1956
150 Werner S C Block R J and Mandl R H Circulating iodoproteins in a nongoitrous adult with primary amenorrhea bony deformities and normal levels of serum precipitable iodine and thyroidal I[131] uptake J Clin Endocrinol 17 1141 1957
151 DiGeorge A M and Paschkis K E Sporadic hypothyroidism associated with goiter J Clin Endocrinol 17 645 1957
152 DeGroot L J Postel S Litvak J and Stanbury J B Peptide-linked iodotyrosines and iodothyronines in the blood of a patient with congenital goiter J Clin Endocrinol 18 158 1958
153 DeGroot L J and Stanbury J B The syndrome of congenital goiter with butanol insoluble serum iodide Am J Med 27 586 1959
153a Derome G Mahaux J and Henry A Iodoprotéinémie anormale chez un enfant porteur d un goitre congénital Ann endocrinol 19 873 1958
154 Litvak J Arnold M B and Stanbury J B La contribucion del laboratorio en el diagnóstico del cretinismo metabólico Medicina 17 181 1957
155 Aballi A J Costales F and Frendes Z Cretinismo esporádico con bocio Rev cubana pediat 27 513 1955
156 Smallpiece V Cretinism with goitre in infancy Lancet 1 565 1949
157 Silverman S H and Wilkins L Radioiodine uptake in the study of different types of hypothyroidism in childhood Pediatrics 12 288 1953
158 Levy R P Kelly L W and Jeffries W McK The effects of thyrotropin and desiccated thyroid upon hypothyroidism with goiter Am J M Sc 231 61 1956
159 Lang L D Preadolescent recurrent nodular goiter in 3 children of the same family Tr Am A Study Goiter 4th Internat Conf 306 312 1939
160 Fletcher K I Litvak J and Stanbury J B The capacity of normal man to deiodinate iodotyrosine Acta endocrinol 29 307 1958
161 Carr E A Beierwaltes W H Raman G Dodson V N Tanton J Betts J S and Stambaugh R A The effect of maternal thyroid function on fetal thyroid function and development J Clin Endocrinol 19 1 1959

## Chapter 10

# Phenylketonuria*

W Eugene Knox

The study of phenylpyruvic amentia may throw light on the whole problem of mental deficiency [1] Now only 25 years after Følling s original description of phenylketonuria [1] this modest statement by the leading investigator of this disease points out the sole major problem left to be solved This chapter aims to collect and codify the considerable knowledge of phenylketonuria in the hope that it will serve as a basis primarily for the future study of the mental defect in phenylketonuria an endeavor which can potentially furnish insight into the nature and mechanism of development of the intellectual functions

## HISTORY AND DEFINITION

Garrod s recognition of the relationship between gene enzyme and clinical abnormality 25 years before the first case of phenylketonuria was described provided the conceptual basis for the disorder [2] Indeed it was the first of these diseases to have the postulates of Garrod unequivocally demonstrated

In 1934 Følling described 10 patients some of them siblings who excreted phenylpyruvic acid and were mentally deficient Jervis [3] then proved that the condition was inherited through a single autosomal recessive gene and showed that large amounts of phenylalanine accumulated in the body of these patients [4] He located the metabolic error as the inability to oxidize phenylalanine to tyrosine [5] In 1953 Jervis demonstrated that the phenylalanine hydroxylase of the liver was inactive in these patients [6] A rational therapy best described as an effective preventive regimen consisting of a low phenylalanine diet was then developed [7 8] Tests for distinguishing the heterozygotes of phenyl

* This work was done under U S Atomic Energy Commission Contract AT(30-1) 901 and Public Health Service Grant A 567

ketonuria followed [9] These salient facts provide the means for understanding and controlling phenylketonuria Many cogent reviews of this disease have appeared [10–15] It has been variously termed *imbecillitas phenylpyruvica* [7] *Følling's disease* and *phenylpyruvic oligophrenia* [7] The term *phenylketonuria* first introduced by Penrose and Quastel [16] has now been generally adopted

The uniform manifestations and precision with which phenylketonuria can be identified allow it to be defined exactly It is required that both parents of a patient have the defective form of one of the two genes controlling phenylalanine hydroxylase An apparent reduction in phenylalanine hydroxylase activity can be detected in the parents but there is no serious consequence of this inadequacy On the average one out of four offspring from two heterozygous parents has both genes defective in these offspring there is no active phenylalanine hydroxylase and phenylketonuria results Both sexes are affected equally often

The disability is first manifest several weeks after birth initially by elevation of plasma phenylalanine to thirty times the normal level and by the excretion of phenylpyruvic acid After 6 months retardation of mental development is evident Seizures and other neurologic abnormalities diluted pigmentation of hair and skin and eczema may occur In older children and adults the process remains stationary but life expectancy is reduced The majority of patients are idiots a few are imbeciles and rare patients have borderline intellectual development

The incidence of the disease is approximately 1 in 25 000 among mixed populations stemming from northern European countries Most of the patients are found in institutions where they make up about 1 per cent of the mentally defective population The disease is readily diagnosed by the olive green color produced in the urine upon addition of $FeCl_3$ This color is due to phenylpyruvic acid

## CHARACTERISTICS

### INCIDENCE

The primary data for calculating the incidence of phenylketonuria consist of its frequency among institutionalized mentally defective individuals Surveys of a total of 48 536 defective patients examined in 12 different countries were compiled by Jervis [12] A total of 312 phenylketonuric patients was found or an average incidence of 0 64 per cent of the world wide institutionalized defective population The problems raised by these data are whether the disease is evenly distributed among peoples and the numerical relation between the defective populations and the populations from which they are drawn The incidence in single institutions varied from 0 per cent in French Canada to 2 7 per cent in England From the figure of 0 64 per cent as the incidence of phenyl

ketonuria in the defective population and an estimate of somewhat less than 1 per cent as the incidence of defectives in the general population (estimated for the United States), Jervis calculated that the incidence of phenylketonuria in the general population was between 2 and 6 per 100 000 He accepted 4 per 100 000 as the order of the real figure It is clear that better estimates of the incidence could be obtained only for specific populations

Other local and independent estimates of the incidence of phenyl ketonuria are therefore of interest Munro [17] calculated the incidence in England to be between 2 and 6 per 100 000 For a south Swedish region comprising nearly half the Swedish population Larson [18] similarly calculated an incidence of 3 5 per 100 000 There were first-cousin marriages among 12 5 per cent of the parents of the Swedish phenyl ketonuria probands From Larson s [19] later estimate of 1 7 per cent frequency of first cousin marriages in this population a similar incidence of phenylketonuria can be calculated

Methods which approach complete ascertainment in localized popula tions have given closely similar figures A C Stevenson s unpublished data for the incidence in Northern Ireland were 10 per 100 000 at birth and 3 per 100 000 in the living population Armstrong and Low [20] cal culated an incidence of 1 in 20 400 in Utah They noted that all except 3 of the 18 phenylketonuric persons born in Utah and known to them were under 16 years of age They suspected that this unusual age dis- tribution might be due to a high death rate among phenylketonuric patients It should be noted that Larson s [18] estimate of the incidence of phenylketonuria in Sweden included a correction for the higher mor tality among low grade defectives It can be concluded that a varying incidence of from 3 to 5 per 100 000 occurs in populations of North European origin

The survey of Jervis [12] indicated that phenylketonuria was most common in Northern European or Scandinavian countries and in popu lations derived from this stock The average was heavily weighted by the high proportion of surveys from such countries The incidences therefore hold only for countries of this population make-up In individual series of case from such countries as the United State it was found that certain segments of the population contributed far fewer phenylketonuric persons than expected from their numerical representation in the general population Jervis found no phenylketonuric patients among 1 000 mentally defective Jews and only one whose parents were Negro-Indian and Negro-White Jews and Negroes were common among the mentally defective population studied Italians on the other hand made up about 18 per cent of both the phenylketonuric patients and the institutional population tudied [21] The handful of exceptional cases not of Nordic origin which have been reported are tabulated in Table 10 1 These

cases indicate that the disease is exceptionally rare but is not unknown in Jewish, Negro and Japanese populations. Two patients described by Bhaskaran [28] in the *Indian Medical Gazette* were Canadians.

It should be noted that an incidence of phenylketonuria of about one in 25 000 in the general population is of the same order of magnitude as that of other rare inborn errors of metabolism like alcaptonuria, cystinuria and albinism. Yet the number of cases reported in only 20 years was 313 in 1954 [12]. With the interest aroused since then by treatment of this

TABLE 10-1 NON-NORDIC CASES OF PHENYLKETONURIA

| Origin | No. of individuals | Reference |
|---|---|---|
| Spanish | 1 | [illegible] |
| Jewish | [illegible] | [3] |
| Mexican | 1 | [11] |
| Negro | | |
| Indian-white | 1 | [9] (case 98) |
| Brazilian | 1 | [4] |
| Mulatto | 1 | [illegible] |
| Japanese | 8 | [7] |
| | | [illegible] |

disease, the total of reported cases is approaching 1 000. This is a considerably larger number than the total cases reported over many years of the other rare hereditary diseases.

Larson's estimate of 3.5 phenylketonuric persons per 100 000 include 1.1 per 100 000 drawn with relatively high incidence (0.76 per cent) from idiots and imbeciles (who make up only 0.15 per cent of the population) and 2.4 per 100 000 drawn with relatively low incidence (0.16 per cent) from the more frequent morons in the population (1.5 per cent). Since the latter group is incompletely institutionalized and since it contribute the largest absolute number of phenylketonuric patients, there may be many noninstitutionalized persons with phenylketonuria. A study of 8 220 individuals not known to be mentally defective did not reveal any cases of phenylketonuria [29], nor did a survey of some 10 000 young healthy men in military service [12]. But among morons Larson [18] found 2 in 1 277, and Schonenberg et al. [30] 1 in 2 016. Gibbs and Woolf [30a] found 1 in a survey of 1 141 normal infants. Although a large absolute number of unrecognized phenylketonuric persons might be expected in the general population, they would be so rare (<2.4 per 100 000) that only enormous surveys could find them. It would appear from the available figures that the absolute number of known cases of phenylketonuria could perhaps be doubled by recognition of all cases with higher grade intellectual function.

AGE DISTRIBUTION AND CAUSE OF DEATH

The age distributions of all sizable series of patients show deficiencies in the numbers of those under 5 years and of those over 35 years of age The former reflects tardy diagnosis plus a period when patients may be kept at home since the majority of reports come from institutions The scarcity of older patients may be only the result of the higher death rate common to institutionalized persons and particularly to those who are mentally defective The graphed age incidence curves of phenylketonuric

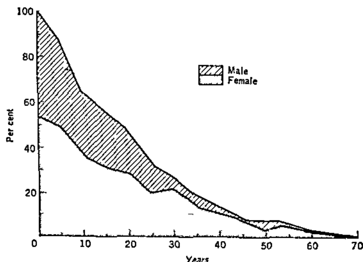



Fig 10 1 Age and sex distribution of phenylketonuric patients (*by permission of K Lang [13]*)

patients constructed by Lang [*13*] from the ages of 500 reported patients (Fig 10 1) indicate that half died by 20 years of age and three quarters by 30 years of age A group of 34 patients from Massachusetts included 2 over 50 years and 2 who were 60 years of age [*31*] Alvord et al [*32*] performed an autopsy on a 67 year-old patient Lang [*13*] referred to a 68-year old patient Hassel and Brunsting [*32a*] studied a 72 year old patient There has been no study to find if the life span of phenylketonuric patients is shortened in a specific way except for Lang s evidence that males die earlier than females

The ages and causes of death of 26 patients are available from the autopsy reports given in Table 10-2 plus a few others mentioned casually in the literature [*6 10 31 33*] These are summarized in Table 10-3 Most of the reported deaths were in young individuals The majority died from infectious diseases that are particularly prevalent among institutionalized populations

TABLE 10-2 PATHOLOGIC ANATOMY OF PHENYLKETONURIA—AUTOPSY LIST

| *Age yr* | *Sex* | *Mental state* | *Cause of death* | *Central nervous system* | *Reference* |
|---|---|---|---|---|---|
| 1 5/12 | M | Severely retarded | Staphylococcic septicemia 1 yr illness | Marked lack of myelination | [39] |
| 1 11/12 | F | Severely retarded | Vomiting and epileptic crisis old tuberculosis | Lack of myelination excessive gliosis | [33] |
| 3 | M | | | Paleness of myelin in temporal regions | [12] |
| 5 1/2 | F | Idiot | Interstitial pneumonia | Areas of deficient myelination | [39] |
| 9 7/12 | M | Idiot | Bronchopneumonia | Cerebral edema | [34] |
| 12 | F | Idiot | Cachexia pyrexia liver degeneration | One small necrotic spot in frontal white matter | [35] |
| 14 | | | | Hypopigmented basal ganglia | [36] |
| 15 | M | Idiot | Intestinal obstruction | Slight paleness of myelin in corticopontine tracts and hyperplasia of macroglia | [37] |
| 18 | F | *Idiot* | *Bronchopneumonia* liver disease | *No gross pathologic* change | [38] |
| 20 | F | Idiot | Perforated ventricular ulcer | Dense cytoarchitecture in deepest cortical layers | [37] |
| 21 | F | Idiot | Pulmonary and abdominal tuberculosis | No significant histologic changes | [37] |
| 21 | F | IQ 8 | Pulmonary tuberculosis | Degeneration of white matter throughout brain | [40] |
| 21 | M | IQ *21* | Pulmonary tuberculosis | Degeneration of myelin (pons cerebellum and cord) and deficiency elsewhere | [40] |
| 21 | F | | Cavernous pulmonary tuberculosis | Large medullary cyst (artifact?) | [41] |
| 25 | F | Idiot | Pneumonia | No gross pathologic change | [38] |
| 25 | F | Idiot | Fever—5 days | Intense gliosis of occipital lobes | [19] |
| 25 | | Imbecile(?) | Schilder's disease | Typical lesions | [19] |
| Adult | | | | No demyelination | [12] |
| Adult | | Idiot | Pulmonary tuberculosis | No significant abnormality | [43] |
| Adult | | Idiot | Pneumonia | No significant abnormality | [43] |
| Adult | | | Miliary tuberculosis | Sieve like extrapyramidal centers | [44] |
| Adult | | | | No gross pathologic change brain weight low | [45] |
| 33 | M | Idiot | *Pulmonary tuberculosis* | *Honeycombed center of* optic chiasma myelin loss and gliosis | [39] |
| 54 | | | | Glial excess—astrocytes | [39] |
| 67 | | | Thyroid carcinoma | Glial excess—astrocytes | [39] |
| | | | Brain biopsy | Normal cells and tissues | [46] |
| | | | Brain biopsy | Normal cells and tissues | [46] |

TABLE 10-3 CAUSES AND DECADE OF DEATH IN PHENYLKETONURIA

| *Decade* | *No of deaths* | *Cause* | *No of deaths* |
|---|---|---|---|
| 1 | 8 | Tuberculosis | 8 |
| 2 | 4 | Pneumonia | 6 |
| 3 | 10 | Liver disease | 3 |
| 4 | 1 | Nephritis | 3 |
| 5 | 0 | Perforated ulcer | 2 |
| 6 | 2 | Septicemia | 1 |
| 7 | 1 | Carcinoma | 1 |
| | | Miscellaneous 1 each | 2 |
| Total | 26 | Total | 26 |

SEX

The difference in death rates probably is the reason why numerous small series in the literature have shown a slight excess of female over male patients There was no difference between the sexes in the massive compilation by Jervis [12] 237 females (51 per cent) and 228 males (49 per cent) The characteristics of the disease in the two sexes have not been compared

INTELLIGENCE

*Deterioration*

Patients with phenylketonuria not infrequently lose accomplishments they once had in childhood This should not be interpreted as evidence for a progressive disease process after infancy Paine [47] had the following to say about this question

> Cessation of independent locomotion however is by no means rare in very low grade retarded patients and it is quite common for very retarded children at one stage to reproduce a parrot like speech which is later lost This repeating of words spoken to the child by parents is perhaps more akin to a conditioned trick such as playing pat-a-cake or waving goodbye and may be lost if it is not later converted into the use of words to convey meaning rather than merely to win approval

He also mentions that early estimates of intelligence inevitably depend on motor development which is likely to be less delayed than the acquisition of speech and other interests or abilities after infancy A young patient therefore appears proportionately less retarded in infancy than in later childhood Institutionalization itself can also account for the apparent deterioration of some patients Others have concurrent organic brain disease (see Pathologic Findings below and Table 10-2) Nevertheless a real deterioration magnified by the failure of expected capabilities

to develop often occurs shortly after 6 months of age when the severe neurologic disturbances appear

*Distribution*

The intelligence quotients of phenylketonuric patients in two independent studies of institutionalized patients are shown in Fig 10-2 [12, 47] The great majority are idiots (I Q <20) and the remainder nearly all imbeciles (I Q <50) Many cannot walk, talk or control their excretory sphincters

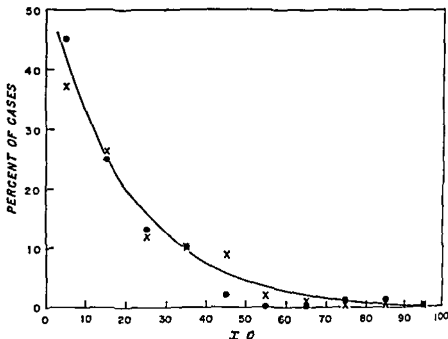



Fig 10-2 Frequency distribution of I Q s among 330 patients [1 ] and 101 patients [31] The total of – per cent above I Q 60 would account for –0 such patients in a total of 1 000 Table 10-4 lists the 16 such patients who are known

Lang [13] noted that the intelligence of phenylketonuric patients over the age of 10 years is higher on the average than at younger ages because of the earlier deaths of the most severely affected ones Larson s examination of 1 277 institutionalized morons revealed only 2 phenylketonuric patients (0 16 per cent) as compared with a fivefold higher incidence among idiots and imbeciles (0 76 per cent) Schönenberg et al [30] found only 1 phenylketonuric patient among 2 016 students in special schools for morons Woolf and Vulliamy—cited in [49]—found only 1 case among 2,200 educationally subnormal children (I Q 50 to 70) These surveys total 4 in 5 493 subnormal persons (0 07 per cent) and establish that the

general shape of the distribution curve is not an artifact arising simply from more frequent hospitalization of the more severe defectives The trend of the I Q distribution curve of known cases emphasizes that high grade defectives must be rare Probably less than 2 per cent of all phenyl ketonuric patients would be expected to have I Q 's above 60

*High-grade Phenylketonuric Mental Defectives*

The subtlety of the factors making for the intelligence of the human mind is nowhere better shown than in the occurrence of these few reason ably competent patients with a disease that almost invariably produces idiots or imbeciles Their existence is the best argument against the term ' phenylpyruvic oligophrenia ' Although none of the high grade cases reported is intellectually normal their superiority to the vast majority of phenylketonuric patients is most interesting

A total of 16 high grade (intellectually ' borderline or dull and back ward ) phenylketonuric patients has been reported They are listed in Table 10-4 To these perhaps should be added Cases 31 and 34 of Følling

TABLE 10-4 HIGH GRADE UNTREATED PHENYLKETONURIC PATIENTS

| No | Age y | Sex | I Q | Phenylpyruvic acid (urine) | Phenylalanine (plasma) µmoles/ml | Reference |
|---|---|---|---|---|---|---|
| 1 | 6/12 | F | 8[illegible] | 0 8 1 5 gm/gm creatinine | 1 15 1 88 | [[illegible]0] |
| 2 | 3 | F | [illegible]1 | Positive | | [51] |
| 3 | 6 | F | 91 | 0 0168 gm/hr | 0 6[illegible] (average of 4) | [5[illegible]] |
| 4 | 6 4/12 | M | 103 | 0 14 –0 07 gm/day | 0 3 (fasting)<br>1 0[illegible] (post cibum) | [49] |
| 5 | 7 | M | 86 | Positive | | [1[illegible]] |
| 6 | | | [illegible]0 | Like defective sibling I Q <[illegible]0 | | [[illegible]3] |
| 7 | 7 2/12 | F | 6 | FeCl test stronger than in idiot sister | | [54] |
| 8 | 7 8/12 | M | [illegible]6 | 0 5 gm/day (see text) | | [33] |
| 9 | 7 11/12 | F | 5 | Positive | | [[illegible]] |
| 10 | 8 | F | [illegible]1 | 0 5–0 65 gm /day | 2 3 [illegible]9 | [5[illegible]] |
| 11 | 8 3/12 | F | [illegible]0 | Positive | | [5[illegible]] |
| 1[illegible] | 1[illegible] | M | 8[illegible] | 31 mg/100 ml | | [5[illegible]] |
| 13 | 13 | F | 69 | 1 0[illegible] gm/gm creatinine | 1 81 | [58] |
| 14 | [illegible]9 | M | 90 | Positive | | [58] |
| 15 | 35 | M | 69 | Positive | | [[illegible]3] |
| 16 | 50 | M | 67 | 0 2–0 3 gm/gm creatinine (sometimes negative ) | 1 33 | [50] |

Mohr and Ruud [10] Case 3 of Armstrong and Tyler [8] and Case 21 of Wright and Tarjan [1[illegible]] making a total of 20 The number is not greater than expected from the distribution of intelligence already discussed The number of these cases helps to establish an incidence of

approximately 2 per cent with I Q above 60 This value can be used as a baseline to measure the effects of treatment on a group of phenylketonuric patients (see Treatment later in this chapter)

Several additional cases are described [9 10 59] who less certainly belong in the high grade group

A compilation of these high grade phenylketonuric patients has disclosed that no serious neurologic defects occurred although the usual EEG abnormalities were found in several patients and one may have had seizures Only in two was pigmentation more marked than expected None could be described as normal All had some degree of intellectual defect or emotional lability and minor neurologic changes such as hyperactive reflexes rigid gait or tremors The mild organic defects and abnormal behavior of these high grade patients reemphasize the evidences for a dissociated psychic development described by Bickel and Grüter [52]

The metabolism of phenylalanine by these patients deserves to be studied Metabolism through alternate pathways must be considered In only 4 of the 16 reported patients was there evidence that the biochemical defect was milder than in the usual phenylketonuric patient In 4 other well studied patients the biochemical defect was as severe as in typical patients In the remaining 8 no evidence is available for estimating degrees of biochemical defect Thus it is not established that a milder biochemical defect occurs in any of these patients

There is no obvious genetic explanation for the occurrence of high grade phenylketonuric patients Eight had severely affected phenylketonuric siblings The son of another was severely affected Only in one family was there a second relatively high grade patient A variant (allelic) gene with a mild mental effect in these particular families is accordingly not an explanation Having intellectually superior parents also fails to explain the difference in the degree of defect in sib pairs

Heterozygosity is not a tenable explanation of the intelligence of some of these patients The pedigrees within this group except for one father son pair are typical of a recessive trait There were no positive $FeCl_3$ tests on parents to indicate an incompletely recessive disease The highest plasma phenylalanine concentration of a heterozygote on record is 0 20 μmoles per ml [62] which is well below the level of 0 9 μmoles per ml hypothesized as the threshold for excretion of significant amounts of phenylpyruvic acid [20 50]

Environmental influences or modifying genes would appear to offer the most reasonable explanation for the high grade patients Birth order seems to be a factor since at least 6 of the 8 patients with defective sibs were born after the defective sib Birth order is unknown in the other 2 cases In only 1 family was a defective child born after the high grade phenylketonuric child but even here another defective child was the first born [27]

It has been suggested that poor reabsorption of phenylalanine by the kidney tubules might lower the concentration of metabolites in the blood and tissues or alter the pattern of metabolism of the toxic metabolites There is no evidence for such physiologic differences in the known high grade phenylketonuric patients Altered carbohydrate metabolism has also been suggested Of the 5 phenylketonuric sibs reported by Penrose [61] the 3 most intelligent were also diabetic or at least had glucosuria (I Q s as high as 55) while the two nonglucosuric phenylketonuric sibs were idiots

It is to be expected that the degree of mental deficiency being a remote consequence of the reduced activity of an enzyme would vary much more than the degree of the enzyme defect The existence of this group of high grade phenylketonuric patients does not support the view that the biochemical defect is causally unrelated to the mental defect nor does it suggest that the biochemical and mental defects are pleiotropic effects of the same gene These cases do permit one to see the specific kind of intellectual damage done in its least complicated form They also offer the encouragement that real improvement can be attained with rational therapy

## CLINICAL FEATURES

Useful and independent clinical descriptions are provided by the reports of 50 cases by Jervis [21] 41 by Følling Mohr and Ruud [10] 15 by Cowie [38] 21 by Wright and Tarjan [15] and 106 by Paine [47] The

TABLE 10-5 MAJOR CLINICAL FINDINGS IN PHENYLKETONURIA

| *Finding* | *Incidence per cent* | *Occurrence in low-grade patients* |
|---|---|---|
| Agitated behavior | 90–32 | + |
| EEG abnormalities | 80 | –† |
| Muscular hypertonicity | 75 | – |
| Microcephaly | 68 | + |
| Hyperactive reflexes | 60 | + |
| Blond hair blue eyes | 6 | – |
| Unable to talk | 63 | + |
| Hyperkinesis | 50 | + |
| Unable to walk (and usually incontinent) | 35 | + |
| Tremors | 30 | + |
| Eczema | 10–31 | – |
| Seizures | [illegible] | + |

Percentages of incidences are approximately those reported in the major series adjusted for redefinition of signs as given in text The findings occurring more frequently or severely in the low grade patients are marked +

† The incidence of EEG abnormalities is not different in high- and low-grade patients but the latter may have more obviously abnormal tracings

author has used these as well as those in his own experience as the basis for evaluating smaller series or single cases A tabulation of findings is given in Table 10-5

The clinical descriptions of patients with phenylketonuria are still inadequate A large number of rare characteristics have been described each by one or a few observers and sometimes denied by other authors. These include kyphosis pes planus spina bifida syndactylia of toes intraventricular heart block hypogenitalism dermatographia light sensitivity, hypersegmentation of neutrophil blood cells decreased galactose tolerance, and slightly increased basal metabolism Acrocyanosis or poor peripheral circulation, is frequently mentioned but is probably not peculiar to the disease [62] Many phenylketonuric patients have an indisputable smell variously described as a "musty mousey' wolf like or barny odor It is referable to the phenylacetic acid excreted by these patients This smell may account for the hyperhidrosis often mentioned The allegation that these patients excrete phenylacetic acid in their sweat has not been demonstrated chemically

*Development*

The usual developmental landmarks the times of sitting walking and talking are sometimes reached at normal ages but usually are delayed. At the latest ages at which the performance would normally be expected 35 per cent could not walk and 63 per cent could not talk Sphincter control is absent in most of those who cannot walk Dentition is often delayed until after the eleventh month Blainey and Gulliford [55] found enamel hypoplasia in 3 patients No other developmental abnormalities have been regularly observed

*Appearance*

**Body Size, Weight, and Shape** Children with phenylketonuria are often under standard height and weight for ages and the adults smaller than average [47] Large or overweight individuals are seldom seen It must be remembered that adequate food intake is difficult to achieve in very retarded individuals The general physical development is at least as good as that of nonphenylketonuric individuals of comparable intellectual level Microcephaly is a regular finding in over half the cases [47] It is more marked in the low grade patients Head circumference averages nearly 2 cm smaller than normal [63] Prominent or prognathic maxillae with consequent widening of the interdental spaces have been described as a typical configuration of the skull [15 54 64]

**Stance and Gait.** A stiff gait with short steps a stooping walk' or a stumbling gait is often described A pithecoid stance is sometimes seen and in severely defective patients a tailorwise sitting position or Schneidersitz [54] is typical (Fig 10 3) The abnormalities of

muscle tone contributing to these changes are de cribed with the neurologic signs

**Skin Lesions** Eczema usually beginning in infancy occurred in 19 per cent of Paine s ca es In the majority it persisted to adolescence or adult hood The incidence was 34 per cent in the author s survey of 101 pub li hed cases In one-third the eczema was said to be severe and one



Fig 10 3 The characteristic *Sch eider it* of severely defective phenylketonuric pati nt (*By pe mission of K La g [13]*) Incessant rhythmic motions complete the usu l pi ture [5 ]

patient died with generalized eczema A dry or rough skin was often noted Some of these skin manifestations can be attributed to unhygienic conditions and to a light and en itive skin Følling et al [*10*] emphasized the frequency of the ame affections in normal individuals The one published dermatologic tudy [*32a*] demon trated that phenylketonuric skin was normally tanned by the sun and was not abnormally ensitive to unlight

**Pigmentation** Deficient pigmentation with blond hair and blue eyes is characteristic of phenylketonuria The coloration is not abnormal in

type and was not noted in Følling's original description from Oslo where most individuals have a similar coloration In Paine's cases from the northeastern United States 64 per cent had blue eyes and 17 per cent brown eyes Sixty per cent had blond hair and the remainder light brown or brown Berg and Stern [64] demonstrated a significantly lighter iris color in 26 phenylketonuric mentally defective patients than in controls and lighter irides in 12 phenylketonuric patients than in 23 of their unaffected sib There was no relation to I Q Even though the incidence is highest among Nordic people each patient has a relatively lighter complexion than other members of his family The dilution of hair color in phenylketonuric patients compared with their unaffected relatives has been demonstrated by reflecting spectrophotometry [38] Striking instances of blond phenylketonuric patients in darkly pigmented families of Sicilian [3] or Spanish [22] origin have been reported Another striking case was said to be a mulatto [25] He had sandy blond hair and blue eyes but negroid fundi The physical characteristics of the one other Negro patient were not described [23] Phenylketonuric Japanese patients have brown hair [26 27] The normally pigmented areas of the brain such as the substantia nigra and locus ceruleus may lack pigmentation

*Nervous System*

The vast majority of patients show a series of typical neurologic changes running parallel to the degree of mental defect and in general worse in severely defective individuals Traces of the same abnormalities are evident in the high grade patients

**Epilepsy** A history of convulsive seizures appears in 26 per cent of Paine's series plus the 418 cases reported by Jervis [12] Seizures usually began during the first 18 months of life and stopped spontaneously before adulthood The incidence of seizures was higher and the attacks were more severe in the severely defective patients Recurrent episodes of staring or inattentiveness after 6 months of age are very frequently recorded The incidence of these petit mal attacks plus overt convulsions gives an over all incidence of abnormal cerebral activity which is probably over 50 per cent The epilepsy is amenable to the usual therapy Prolonged severe epilepsy is exceptional but it has been noted as a complication of the low phenylalanine diet therapy

**Electroencephalogram** The majority of phenylketonuria patients have abnormal EEG patterns regardless of whether or not they have had seizures The incidence is approximately the same with low or high grade defective patients Paine [47] found 78 per cent abnormal recordings in 33 cases (75 per cent were abnormal in 24 of these cases who never had seizures) Lois Rosenberg and Gibbs [66] reported 95 per cent abnormal tracings in 19 patients Their investigation included the technique of sleep recording by means of which disorders of this type are best seen

The abnormalities consist of spike and wave complexes and the petit mal variant type (even in some individuals without epilepsy) The most common finding is a mixture of high voltage fast and slow waves occurring more irregularly than in petit mal Fois et al likened the disorganized sleep patterns to those seen in hydrocephalus with injury to the thalamus or hypothalamus These abnormalities are not pathognomonic but they do suggest deep mid line brain damage

**Behavior** The statement of Wright and Tarjan [15] is most apt None could be described as friendly placid or happy They are restless jerky and fearful individuals Their behavior ranges from that of the hyanxious and restless high grade patient to the destructive and noisy psychotic episodes observed in 10 per cent of the patients Night terrors beset the higher grade patients Uncontrollable temper tantrums are common (32 per cent in Paine s series) The hyperactivity irritability and uncontrollable temper are the usual reasons given for admitting these patients to institutions

**Muscular Hypertonicity** Jervis found increased muscle tone in 70 per cent and Wright and Tarjan in 76 per cent The patients seem always ready to jump This unrelaxed attitude may be responsible for the awkward or even rigid short stepped gait with few associative movements that is seen in 20 per cent of the low grade group Approximately 20 per cent are described as having hypotonic muscle tone Jervis also described cog wheel rigidity This finding is particularly difficult to evaluate in these patients

**Hyperactive Tendon Reflexes** Two thirds of all patients show abnormally brisk tendon reflexes and in a sizable fraction ankle and patellar clonus can be elicited Clonus persists continuously in some patients

**Adventitious Hyperkinesis** A great variety of abnormal body movements has been described Each patient has his own favored motions out of the infinite variety possible The important point is his restless movement There are voluntary purposeless and repetitive motions of the whole or a part of the body There may be inconspicuous fiddling movements of the fingers or violent body swinging which continues for hours The persistent ankle clonus may be of this type Each patient has a limited repertoire of these movements but if one motion is suppressed others occur They contribute a great deal to the picture of behavioral unrest and agitation Because of their combination with muscular rigidity they are often described as choreiform or athetoid

**Tremors** A fine rapid and irregular tremor of the outstretched hands was seen in 30 per cent of Paine s series It may become stronger on volition and may spread to other parts of the body Together with increased tendon reflexes and jerky rigidity it is one of the reasons for suspecting extrapyramidal disease in these patients even though true spasticity may not be present

**Other Neurologic Findings** A small percentage of phenylketonuric patients has signs of severe brain disease in association with severe mental defect. These are the patients described by Jervis [21] as the 4 per cent with "stationary severe cerebral palsy with diplegia, contractures and pyramidal signs" and by Paine [47] as 5 per cent with spastic para- or tetraplegia. There are rare reports of positive Babinski or Hoffman signs [27, 35]. The relationship of these findings to phenylketonuria is not clear. The patients may have other concurrent diseases or the signs may represent the ultimate degree of cerebral defect caused by phenylketonuria.

**Pneumoencephalographs** Pneumoencephalographs of patients have shown evidence of diffuse cortical atrophy [21, 67].

*Pathologic Findings*

No relevant pathologic changes occur outside the central nervous system in phenylketonuria. The changes in the central nervous system which have been observed do not account for the mental deficiency. Occasional reports have emphasized nonspecific liver abnormalities or such changes as hypoplasia of the pituitary or gonads. Table 10-2 lists the autopsies that have been performed. Most of the deaths occurred in young individuals after long and debilitating illness that might produce a variety of irrelevant pathologic changes. One frequent finding is that the weight of the brains is about two-thirds of normal.

The first autopsy reported on a phenylketonuric subject disclosed another rare disease, neurofibromatosis, with peripheral nerve tumors but without cutaneous nodules or pigmentation. This incidental finding delayed the recognition that phenylketonuria is an extremely subtle and unsolved problem in neuropathology. The second recorded autopsy was little more helpful, since it was performed on an exhumed body [35].

The complete list of autopsies presented here is more extensive than any previously evaluated. Many of the findings can be attributed to coincidental abnormalities, to the effect of the final illness, or to artifacts. Peters [68] concluded his discussion of the subject: *Aus den wenigen anatomisch pathologischen Befunden lässt sich ein charakteristisches pathomorphologisches Syndrom nicht erkennen. Auch über Pathogenese und Ursache lässt sich an Hand der zentralnervösen Veränderungen kein Schluss ziehen.*

The listing of cases by age (Table 10-2) reveals unexpected support for one vaguely defined pathologic process. An abnormality of myelination in phenylketonuria of the sort first suggested by Alvord et al [32] remains a possibility. This view was extended by Scholz [33]. It is based on the findings in the youngest patients (under 5 years of age). It could account for the negative findings in many of the patients who were past the age at which the abnormalities could be clearly seen without detailed

cytopathologic studies Scholz hypothesized that there is a slowing and possibly an inhibition of myelination At the same time glial cells especially those concerned with myelination develop normally or excessively This picture is seen only in the youngest patients The ultimate result in older patients would be an intensive gliosis of oligodendrocytes and astrocytes with more or less normal appearing myelin Pathologic evidence for the deficient myelination is available in all the youngest patients studied and in some of the older patients there is evidence of gliosis although often with an apparently normal degree of myelination The best studied cases are the ones which support this picture of the disease process Myelination normally occurs during the first few years of life during the period when the intellectual defect in phenylketonuria is established

Deficient myelination is well described in younger subjects The youngest child examined was 1½ years of age [3?] The brain showed a marked lack of myelination of the optic cortical cerebellar and spinal tracts The over all extent of myelination was between that found in an 8 month-old fetus and a 2 month-old child The next oldest died at 1 11/12 years in an acute crisis [68] She had been treated with a low phenylalanine diet for 5 months (see Treatment further on) There was a lack of myelination in the optic nerve and tract and in other regions of the brain and spinal cord Myelination was equivalent to that of a 5-month-old infant There were excessive oligodendrocytes and astrocytes which increased the consistency of the brain Paradoxically the gliosis increased in a caudal direction A 3 year-old child was stated only to have some paleness of myelin in the temporal regions [12] Another was 5½ years old [39] The brain also showed a patchy deficiency of myelin which was less marked than in the younger patient observed by the same authors A 3½ year-old not included in Table 10-2 died in hypoglycemia after chronic malnutrition during 2 months treatment with a low phenylalanine diet The only neuropathologic changes were acute and consistent with hypoglycemic encephalopathy [69]

Older children and adults may or may not show findings consistent with the younger patients The paleness of myelin and gliosis in the 15-year-old patient of Leoni [37] is suggestive The 33 year-old patient of Alvord et al [3 ] had myelin loss in the center of the optic chiasma and an intense honeycomb-like gliosis surrounding the empty spaces The patient of Sander [44] had a sieve-like process in the extrapyramidal centers The 25-year-old patient of Corsellis [49] showed an intense gliosis of the occipital lobes but only a few small spots of demyelination perhaps referable to epilepsy early in life The uncommonly dense cytoarchitecture in the cortex of Larson s [33] 70-year-old patient should also be mentioned It is possible that additional cases showing destruction of white matter could be fitted into the still hypothetic pathologic process

Chemical analysis of these brains might provide valuable insight into the cause of defective myelination This process which involves the elaboration of several types of complex lipids and their lamination in lipoprotein layers about the nerve fiber is complicated [81] Myelin is not a simple chemical substance but a complex deposition of lipid largely

**Other Neurologic Findings** A small percentage of phenylketonuric patients has signs of severe brain disease in association with severe mental defect. These are the patients described by Jervis [21] as the 4 per cent with "stationary severe cerebral palsy with diplegia, contractures and pyramidal signs," and by Paine [47] as 5 per cent with spastic para- or tetraplegia. There are rare reports of positive Babinski or Hoffman signs [27, 38]. The relationship of these findings to phenylketonuria is not clear. The patients may have other concurrent diseases, or the signs may represent the ultimate degree of cerebral defect caused by phenylketonuria.

**Pneumoencephalographs** Pneumoencephalographs of patients have shown evidence of diffuse cortical atrophy [21, 67].

*Pathologic Findings*

No relevant pathologic changes occur outside the central nervous system in phenylketonuria. The changes in the central nervous system which have been observed do not account for the mental deficiency. Occasional reports have emphasized nonspecific liver abnormalities or such changes as hypoplasia of the pituitary or gonads. Table 10-2 lists the autopsies that have been performed. Most of the deaths occurred in young individuals after long and debilitating illness that might produce a variety of irrelevant pathologic changes. One frequent finding is that the weight of the brains is about two-thirds of normal.

The first autopsy reported on a phenylketonuric subject disclosed another rare disease, neurofibromatosis, with peripheral nerve tumors but without cutaneous nodules or pigmentation. This incidental finding delayed the recognition that phenylketonuria is an extremely subtle and unsolved problem in neuropathology. The second recorded autopsy was little more helpful, since it was performed on an exhumed body [35].

The complete list of autopsies presented here is more extensive than any previously evaluated. Many of the findings can be attributed to coincidental abnormalities, to the effect of the final illness, or to artifacts. Peters [68] concluded his discussion of the subject: *"Aus den wenigen anatomisch pathologischen Befunden lässt sich ein charakteristisches pathomorphologisches Syndrom nicht erkennen. Auch über Pathogenese und Ursache lässt sich an Hand der zentralnervösen Veränderungen kein Schluss ziehen."*

The listing of cases by age (Table 10-2) reveals unexpected support for one vaguely defined pathologic process. An abnormality of myelination in phenylketonuria of the sort first suggested by Alvord et al. [32] remains a possibility. This view was extended by Scholz [33]. It is based on the findings in the youngest patients (under 5 years of age). It could account for the negative findings in many of the patients who were past the age at which the abnormalities could be clearly seen without detailed

porated into the body protein of the different cells Phenylalanine is also oxidized in the liver to tyrosine The reverse reaction does not occur

Tyrosine undergoes further reactions in various specialized tissues The largest dissimulative reaction of tyrosine occurs in the liver there is a small reaction in the kidney This is oxidative ring fission to components of the citric acid cycle In this way tyrosine is eliminated as an aromatic compound The amount of tyrosine handled in other ways is

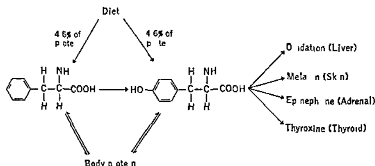



Fig 10-4 Diagram of normal metabolic flow of phenylalanine and tyrosine and the location of the reaction blocked in phenylketonuria ( )

very much smaller though no exact quantitative comparisons can be made These smaller amounts of tyrosine are metabolized in specialized organs such as the skin adrenal glands and thyroid

*D Phenylalanine Metabolism*

The unnatural form of phenylalanine like most other D amino acids is oxidatively deaminated by D amino acid oxidase This reaction forms phenylpyruvic acid which is identical with the typical metabolite of phenylketonuria Since the further metabolism of this compound is rate limited the administration of modest amounts of DL-phenylalanine is followed by the appearance of phenylpyruvic acid in the urine This has been known since the early work of Kotake Masai and Mori [73] it was confirmed by Chandler and Lewis [74] and on it was based one of the first theories of the nature of phenylketonuria Instead of absence of a normal enzyme of metabolism this theory suggested an abnormal enzyme (a racemase ) which converted L- to D phenylalanine which in turn gave rise to the phenylpyruvic acid [10] However Følling found only L-phenylalanine in blood and urine in phenylketonuria and this was confirmed by the microbiologic assays of Prescott et al [75] This route of phenylpyruvic acid formation may confuse experiments when DL-phenylalanine is used but it is otherwise unrelated to the reactions primarily involved here

sphingomyelin and phosphatidylethanolamine in addition to proteolipids cholesterol and protein [70] It is premature to suggest a direct connection between phenylalanine and tyrosine metabolism and this complex chemical event but interference with this process by the abnormal chemical milieu of the phenylketonuric brain is quite possible

**Associated Conditions** Phenylketonuria has been associated with other rare conditions but usually only in single cases Some of these may be other recessive conditions that show up in the same matings which permit phenylketonuria It has been associated with Gowers s muscular dystrophy neurofibromatosis and Schilder s disease To these can be added the association with craniolacunia [71] and with a second metabolic defect characterized by *α* hydroxybutyric acid excretion [72] There is no indication that such associations are other than casual

## BIOCHEMICAL ABNORMALITIES

The normal oxidation of L-phenylalanine to tyrosine in the liver is almost completely stopped in phenylketonuria The evidence is quite *complete that hereditary inactivity of the enzyme for this reaction* phenylalanine hydroxylase is the primary phenotypic lesion in this disease The failure to convert the essential amino acid phenylalanine to tyrosine causes directly and indirectly a host of biochemical physiologic and pathologic repercussions

Not only is there accumulation of the phenylalanine which is constantly fed into the organism by the diet and which must seek the other avenues of removal (and a relative deficiency of tyrosine which becomes an essential dietary amino acid) but a variety of intracellular systems are called upon to adjust their function to this new metabolic state The renal tubular transport system which normally reabsorbs relatively small amounts of phenylalanine is faced with the reabsorption of amounts approaching its maximum transfer capacity Amino acids that are normally concentrated in cells for purposes of growth and metabolism must continue to be taken up in appropriate amounts despite the high concentration of phenylalanine seeking entry Each amino acid residue must continue to be incorporated accurately into the myriad proteins synthesized by the cells regardless of the distorted pattern of free amino acids in the intracellular pool It is remarkable indeed that so few of the functions of the body are seriously impaired in phenylketonuria The relative lack of damage indicates that all processes but one are intact and that biologic processes have great adaptability for meeting new conditions

### PRELIMINARY CONSIDERATIONS

The metabolic flow of phenylalanine and tyrosine appears in Fig 10-4 The amino acids are continuously supplied from the diet and are incor

*The Common Pathway of Amino Acid Metabolism*

Nearly every amino acid has a highly individual pathway by which it is metabolized. Since most amino acids also participate in oxidative deaminations by the L- or D amino acid oxidases and in transaminations to the α keto acids, there is also potentially a common pathway of degradation through the keto acids to the next lower carboxylic acids as shown in Fig 10-5. The major metabolites found in phenylketonuria

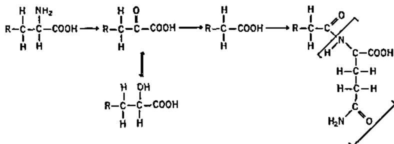



Fig 10-5 A common nonspecific pathway of amino acid degradation through keto and lactic acids, acetic acid, and conjugates of the latter. Such secondary pathways account for many of the metabolites in phenylketonuria

find representation in such sequences of reactions. The parent amino acids are phenylalanine, tyrosine (or its α keto derivative), tryptophan and the hypothetic compound *o*-tyrosine. The classification of the metabolites in phenylketonuria according to a hypothetic series of reactions is illustrated in Table 10 6. Some of the intermediates expected according to this scheme have not been found. The degradation of *o*-tyrosine may occur by another route (through *o*-tyramine) for which enzymes are known to exist in the body. Since this scheme was first presented [*14*] one of the predicted metabolites, indolepyruvic acid, has been added to the list known to be formed in phenylketonuria [*76*]

## BIOCHEMICAL DIAGNOSTIC CRITERIA AND METHODS

The vast majority of phenylketonuric patients have been recognized solely by their excretion of phenylpyruvic acid as identified by the simple $FeCl_3$ test. This single item of qualitative information has been extraordinarily successful in distinguishing phenylketonuria. Diagnosis of crucial cases should be supported by additional chemical criteria. The most valuable of these are increased plasma phenylalanine concentration and the excretion of *o*-hydroxyphenylacetic acid

The $FeCl_3$ test. The addition of several drops of 5 per cent $FeCl_3$ to 5 ml fresh urine first produces some precipitation of phosphates; then as more is added to positive specimen, there appears after 2 or 3 min an olive-

Table 10-6 The Unusual Metabolites in Phenylketonuria

| Parent amino acid | Phenylalanine | o-Tyrosine | Tyrosine | Tryptophan |
|---|---|---|---|---|
| | $C_6H_5{-}CH_2{-}CH(NH_2){-}COOH$ | $o\text{-}HO{-}C_6H_4{-}CH_2{-}CH(NH_2){-}COOH$ | $HO{-}C_6H_4{-}CH_2{-}CH(NH_2){-}COOH$ | $\text{indolyl}{-}CH_2{-}CH(NH_2){-}COOH$ |
| Derivatives | Phenyl = R | o Hydroxyphenyl = R | p Hyd oxyphenyl = R | Indolyl = R |
| -pyruvic | $R{-}CH_2{-}CO{-}COOH$ | $R{-}CH_2{-}CO{-}COOH$ | $R{-}CH_2{-}CO{-}COOH$ | $R{-}CH_2{-}CO{-}COOH$ |
| lactic | $R{-}CH_2{-}CH(OH){-}COOH$ | $R{-}CH_2{-}CH(OH){-}COOH$ | $R{-}CH_2{-}CH(OH){-}COOH$ | $R{-}CH_2{-}CH(OH){-}COOH$ |
| acetic | $R{-}CH_2{-}COOH$ | $R{-}CH_2{-}COOH$ | $R{-}CH_2{-}COOH$ | $R{-}CH_2{-}COOH$ |
| acetyl conjugate | $R{-}CH_2{-}CO{-}NH{-}CH(COOH){-}CH_2{-}CH_2{-}CONH_2$ | $R{-}CH_2{-}CO{-}NH{-}CH_2{-}COOH$ | $R{-}CH_2{-}CO{-}NH{-}CH_2{-}COOH$ | $R{-}CH_2{-}CO{-}NH{-}CH(COOH){-}CH_2{-}CH_2{-}CONH_2$ |

Arranged as products of parent amino acids by reaction of Fig. 10-5. Compounds within the boxes have been identified.

*The Common Pathway of Amino Acid Metabolism*

Nearly every amino acid has a highly individual pathway by which it is metabolized Since most amino acids also participate in oxidative deaminations by the L- or D amino acid oxidases and in transaminations to the α keto acids there is also potentially a common pathway of degradation through the keto acids to the next lower carboxylic acids as shown in Fig 10-5 The major metabolites found in phenylketonuria

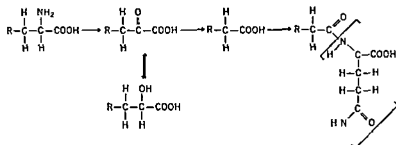



Fig 10-5 A common nonspecific pathway of amino acid degradation through keto and lactic acids acetic acids and conjugates of the latter Such secondary pathways account for many of the metabolites in phenylketonuria

find representation in such sequences of reactions The parent amino acids are phenylalanine tyrosine (or its α keto derivative) tryptophan and the hypothetic compound *o*-tyrosine The classification of the metabolites in phenylketonuria according to a hypothetic series of reactions is illustrated in Table 10 6 Some of the intermediates expected according to this scheme have not been found The degradation of *o*-tyrosine may occur by another route (through *o*-tyramine) for which enzymes are known to exist in the body Since this scheme was first presented [*14*] one of the predicted metabolites indolepyruvic acid has been added to the list known to be formed in phenylketonuria [*76*]

## BIOCHEMICAL DIAGNOSTIC CRITERIA AND METHODS

The vast majority of phenylketonuric patients have been recognized solely by their excretion of phenylpyruvic acid as identified by the simple $FeCl_3$ test This single item of qualitative information has been extraordinarily successful in distinguishing phenylketonuria Diagnosis of crucial cases should be supported by additional chemical criteria The most valuable of these are increased plasma phenylalanine concentration and the excretion of *o*-hydroxyphenylacetic acid

The $FeCl_3$ test The addition of several drops of 5 per cent $FeCl_3$ to 5 ml fresh urine first produces some precipitation of phosphates then as more is added to positive specimens there appears after 2 or 3 min an olive-

green color which fades slowly over the next hour or two Acidification is not necessary

The test is sometimes negative in phenylketonuria when the urinary concentration of phenylpyruvic acid is less than 0 2 mg per ml This occurs in very dilute urine in rare individuals with low phenylpyruvic

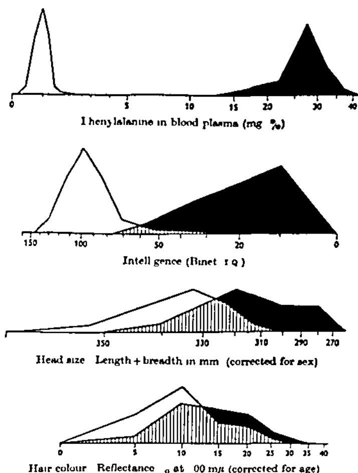



Fig 10-6 Frequency distributions of some characteristic features of phenylketonuria in phenylketonuric patients (*right*) and in control populations (*left*) (*By permission of H Harris [63]*)

acid output with low protein intake or in day old urine after the relatively labile phenylpyruvic acid has decomposed Gibbs and Woolf [*80a*] give 0 05 mg per ml as the limit of detection with ferric chloride The color results from the chelation of $Fe^{3+}$ with the enol group of the phenylpyruvic acid The analogous reaction of $Fe^{3+}$ with acetone is used

is a standard clinical test Acetone acetoacetic acid or salicylic acid gives distinctive colors in this test that are not confused with the typical green reaction of phenylpyruvic acid A green color similar to that given by phenylpyruvic acid occurs with a metabolite of chlorpromazine [77] and at times in urines containing bile Ephemeral green colors caused by p hydroxyphenylpyruvate were found in urines of 7 out of 1 141 infants screened by Gibbs and Woolf [80a] Confirmation of the presence of phenylpyruvic acid is obtained by extracting it from acid solution with ether and repeating the $FeCl_3$ test [78] or by forming the insoluble 2 4 dinitrophenylhydrazone [16] Both tests can be used for quantitative determinations Conditions for increasing the sensitivity of the ferric chloride test were described by Saifer and Harris [78a]

References for the determination of plasma phenylalanine and urinary o-hydroxyphenylacetic acid will be found in the next section

The need for complete reliance upon chemical criteria for the diagnosis of phenylketonuria can be seen from the frequency distribution of certain characteristics of phenylketonuria measured in normal and phenylketonuria populations (Fig 10-6) Only the biochemical criterion furnishes absolute discrimination between normal and phenylketonuric persons even though the two populations differ significantly with respect to certain of the overlapping characteristics

## CHARACTERISTIC METABOLITES

There are no abnormal metabolites in phenylketonuria only normal metabolites in abnormal amounts Følling [1] isolated and identified phenylpyruvic acid as part of the original description of the disease Later [79] he found elevated amounts of L-phenylalanine and probably phenyllactic acid in urine and L-phenylalanine in serum The true significance of the elevated plasma phenylalanine indicating a block in the metabolism of the phenylalanine itself became clear with the confirmation of the high phenylalanine levels by Jervis Block Bolling and Kanze [4] Jervis [5] then demonstrated the block by showing that no rise in blood tyrosine level occurs in the plasma of phenylketonuric patients after a dose of phenylalanine It then became clear that phenylalanine is the primary metabolite which accumulates and that the other metabolites are derivatives of it Later other compounds (derivative of tyrosine and tryptophan) were recognized as arising secondarily from separate metabolic reactions *The compound now known to be* present in abnormal amounts are described below and listed in Table 10-7 10-8

**Phenylalanine** Probably the most accurate plasma phenylalanine concentrations are those determined by column chromatography which gives the lowest values of the several methods Slightly higher values are obtained with the specific enzyme decarboxylase method and still

green color which fades slowly over the next hour or two. Acidification is not necessary.

The test is sometimes negative in phenylketonuria when the urinary concentration of phenylpyruvic acid is less than 0.2 mg per ml. This occurs in very dilute urines, in rare individuals with low phenylpyruvic

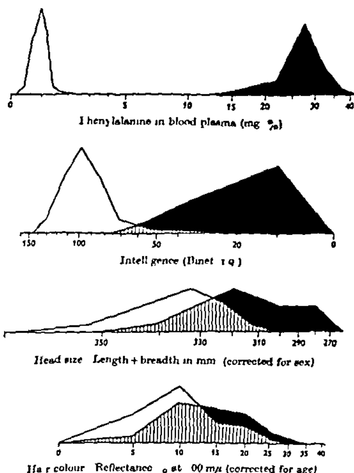



Fig 10-6 Frequency distribution of some characteristic features of phenylketonuria in phenylketonuric patients (*right*) and in control populations (*left*) (*By permission of H Harris [63]*)

acid output with low protein intake or in day old urine after the relatively labile phenylpyruvic acid has decomposed. Gibbs and Woolf [30a] give 0.05 mg per ml as the limit of detection with ferric chloride. The color results from the chelation of $Fe^{3+}$ with the enol group of the phenylpyruvic acid. The analogous reaction of $Fe^{3+}$ with acetone is used

o-tyramine The last a probable precursor of o hydroxyphenylacetic acid [97] is formed from o tyrosine and concentrated in brain and to a lesser extent in liver

*Quantities of Urinary Metabolites*

Many of the values recorded in the literature for the metabolites excreted in abnormal amounts in phenylketonuria are not useful There is great difficulty in collecting all urine for a given period from these patients There is also uncertainty about body size and protein intake of the patients in many reports In Table 10-8 only the urinary excretion values have been included which give a basis for comparison such as the urinary nitrogen or creatinine

**Phenylpyruvic Acid** The phenylpyruvic acid excretions recorded by Jervis [88] have been expressed per 15 gm urinary nitrogen to approximate the daily output of an adult The remarkable constancy within this group which included children as well as adults shows that phenylpyruvic acid excretion in these patients is largely a function of protein intake The larger variation seen in the series of Armstrong and Low [20] which is expressed as per grams creatinine may be the result of varying protein intakes Creatinine excretion reflects body size but not intake although the two are roughly parallel under usual circumstances One patient who excreted only 0 3 gm phenylpyruvic acid per gram creatinine and who was a high grade defective (I Q 67), has been omitted from the second series

**Phenyllactic Acid** Zeller [98] isolated and identified phenyllactic acid from phenylketonuric urine Few measurements have been reported but it is excreted in quantities intermediate between those of phenylpyruvic acid and phenylalanine

**Phenylalanine** Normally phenylalanine is excreted in amounts of 10 to 15 mg per gm creatinine as determined by column chromatography [99] although children under 1 year excrete several times as much of this It can be a sensitive indicator of the blood level if careful measurements are made

**o Hydroxyphenylacetic Acid** Armstrong Shaw and Robinson [91] discovered this unexpected metabolite of phenylalanine in phenylketonuric urine Amounts less than 1 mg per day are normally excreted The excretion of a high grade patient (Case 16 Table 10-4) was the lowest in the series of measurements used for the table (0 08 gm per gm) and has been omitted

**Indolepyruvic, Indolelactic and Indoleacetic Acids** Indolelactic and indoleacetic acids were discovered in abnormal amounts in phenylketonuric urine by Armstrong and Robinson [92] and confirmed by Jepson [100] Schreier and Flaig [7(] completed this group with the discovery of the very labile pyruvic acid derivative These arise from tryp-

higher ones with the microbiologic and paper chromatographic method Accepted normal values are under 0 1 µmole per ml (1 65 mg per 100 ml) Much higher concentrations in normal persons are given by the Kapeller Adler chemical method (0 18 to 0 30 µmoles per ml or 3 to 5 mg per 100 ml) which has frequently been used to measure the high levels found in phenylketonuric patients [59] It is not recommended for the lower normal values [94 95]

Plasma phenylalanine concentrations in heterozygotes of phenylketonuria are definitely higher than those in normal persons as determined by the same method but the difference is small and demonstrable only with the most precise methods of analysis

The markedly elevated plasma phenylalanine in phenylketonuria has been measured by column chromatography in only one patient but this agreed well with the means of the series measured microbiologically and with the enzyme method Several series of determinations in phenylketonuria by the Kapeller Adler method are included in the table This method though admittedly inaccurate in this range [59] is said to give internally consistent results With this method higher phenylalanine values were found in phenylketonuric children under 2 years of age as is shown in the table Other methods have not shown such striking differences in young children

The phenylalanine level in cerebrospinal fluid is approximately one-fourth that in the plasma and the level in sweat is still lower

**Phenylpyruvic Acid** Jervis [89] was able to measure phenylpyruvic acid in the plasma of phenylketonuric patients but he was unable to detect it in normal plasma The low concentration in plasma is referable to renal excretion which may occur in part by active tubular secretion The evidence for this is that probenecid an inhibitor of renal transport decreased the renal excretion of phenylpyruvic acid by one-half and simultaneously increased the blood level of this compound [89] The compound may also be actively secreted by the skin since the concentration of phenylpyruvic acid in sweat is substantially higher than in plasma

**Other Compounds in Blood Phenylacetic Acid, 5 Hydroxytryptamine, Epinephrine** Jervis [12] mentioned that phenylacetic acid is present in the blood of phenylketonuric patients but the amount must be very low Most of the other metabolites formed in abnormal amounts by phenylketonuric patients have been detected only in urine Mention should be made of the pharmacologically active substances which occur in concentrations an order of magnitude lower than those described These include 5 hydroxytryptamine which is lower than normal in the plasma in phenylketonuria (inferred from measurements on serum from incubated clotted blood) [90] epinephrine which is lower than normal in the plasma of all mental defectives [96] and the still hypothetic intermediate

of phenylacetylglutamine Stein Paladini Hirs and Moore [102] reported that 250 to 500 mg per day are excreted by normal individuals and that this accounts for one half of the conjugated glutamic acid in urine They examined by column chromatography one patient with phenylketonuria who excreted 2 4 gm per day Excretions between 300 and 450 mg per day can be calculated from data on two phenylketonuric children studied by Meister Udenfriend and Bessman [103] Coates et al [49] reported figures of 385 to 640 mg per day in their patient with a relatively high I Q

TABLE 10-8 AMOUNTS OF URINARY METABOLITES IN PHENYLKETONURIA

| Compound | N | Mean | Range | Reference |
|---|---|---|---|---|
| Phenylpyruvic acid | 20 | 2 13 | 1 68– 72 gm/15 gm N | [99] |
| | 27 | 2 27 | 0 8–5 6 gm/gm creat | [100] |
| Phenyllactic acid | 20 | 0 95 | 0 60–1 35 gm/15 gm N | [99] |
| Phenylalanine | 20 | 0 45 | 0 30–0 75 gm/15 gm N | [88] |
| o-Hydroxyphenylacetic acid | 12 | 0 22 | 0 10–0 40 gm/gm creat | [91] |
| Indolepyruvic acid | 3 | | 0 11–0 17 gm/day (normal = 0 04 gm/day) | [70] |
| Indolelactic acid | 15 | | 0 0?–0 15 g /gm creat | [92] |
| Indoleacetic acid | | | Amount not given | |
| 5-Hydroxyindoleacetic acid | | | | |
| Other metabolites | 9 | 4 9 ± 0 7 (S E ) | 1 6–8 0 mg/gm creatinine | [90] |
| Phenylketonurics | 9 | 2 9 ± 0 2 (S E ) | 1 6–3 8 mg/gm creatinine | [90] |
| Phenylacetylglutamine | 6 | | 0 03 2 4 gm/day | See text |
| Phenylacetic acid | | | Amount not given | [99] |
| p-Hydroxyphenyllactic acid | | | Trace reported | [55] |

Other compounds that which had been detected in this table p-hydroxyphenylpyruvic acid and p-hydroxyphenylacetic acid

Unpublished determinations by column chromatography done in the author s laboratory by C D Thron gave 760 mg per gm creatinine for the high grade phenylketonuric patient described by Hsia et al [58] and 690 mg per gm creatinine for the severely retarded patient studied in comparison

**p Hydroxyphenylpyruvic, -lactic, and -acetic Acids** These metabolites similarly to the indole acid arise secondarily from tyrosine metabolism The compounds may be detected by paper chromatography and the amounts excreted are not known

## *Dynamic Interrelationships of Metabolites*

Certain correlations have been noted among the quantities of the various metabolites in phenylketonuria The dietary treatment of phenylketonuria has made it clear that the plasma level of phenylalanine reflects the amount in the diet So does the amount of phenylpyruvic acid excreted as already deduced from its constancy in relation to urinary nitrogen Consequently there is a rough correlation of the serum phenyl

tophan metabolism Indoleacetic acid is said to be excreted in larger amounts than indolelactic acid, but no figures are available [92]

**5-Hydroxyindoleacetic Acid** 5 Hydroxyindoleacetic acid excretion was first found to be slightly low in phenylketonuria by Armstrong and Robinson [92] This was confirmed by Ferrari Campagnari and Guida

TABLE 10-7 BLOOD CONCENTRATIONS OF METABOLITES

| *Metabolite* | *No of patients* | *Mean ± SD μmol /ml* | *Range mol e/ml* | *Method* | *Reference* |
|---|---|---|---|---|---|
| Ph nyl l | | | | | |
| N rm l | | | | | |
| Pl ma | 14 | 0 051 | 0 040–0 0 4 | C l mn chromat graphy | [ ] [41] [4] |
| Plasm | 106 | 0 078 ± 0 015 | 0 040–0 115 | E yme | [7] |
| Plas | 17 | 0 0 8 ± 0 003 (S E ) | 0 030–0 079 | M rol l gy | [ 5] |
| | | 0 084 ± 0 019 | | M c l l gy | [95] |
| Plasma | 17 | 0 1 0 ± 0 0 4 | 0 067–0 240 | M c l l gy | [94] |
| Pl ma | 10 | 0 09 ± 0 035 | 0 0 1–0 151 | P per chr matog phy | [45] |
| H t o yg te | | | | | |
| Pl | 2 | 0 103 ± 0 016 | 0 075–0 205 | En yme | [64] |
| Plasm | 10 | 0 151 ± 0 034 | 0 095–0 254 | Paper hromat graphy | [95] |
| Ph ylk t n | | | | | |
| Plasm ( ) | 1 | 1 42 | | Col m chr m t graphy | [98] |
| | 18 | 1 68 | 1 15–2 1 | M l gy | [ ] |
| Plasm (ser m) | 34 | 1 78 | 1 03 81 | E ym | [51] |
| Plasm (se ) | 12 (<2 y ) | 4 05 | 18–6 00 | K pell Adl r | [ ] |
| Plasma (serum) | 4 (>2 y ) | 48 | 1 33 3 21 | Adl | [ ] |
| Plasm (se m) | 18 ( ll ges) | 54 | 1 33 3 82 | Adl r | [ ] |
| S n l fl l | 9 | 0 44 | 0 37–0 50 | M l gy | [ ] |
| S t | 10 | 0 70 | 0 093–0 109 | M r l l gy | [ ] |
| Phe ylpyr c d | | | | | |
| Ph ylk t n | | | | | |
| Plasma | 39 | 0 043 ± 0 015 | 0 019–0 108 | FeCl t | [89] |
| S t | 10 | 1 55 | 0 30–3 40 | Pl ylhyd z | [99] |
| 5 Hyd ytrypt m (se m) | | | | | |
| Oth m nt l d fi y | 9 | 270 ± 51 (S E ) (mg/ml) | 77 608 | Ut as y | [90] |
| Ph ylk t | 9 | 57 ± 11 (S E ) ( g/ l) | 8 1 0 | Ut assay | [90] |

[101] The measurements shown in the table by Pare et al [90] accompanied the demonstration of low serum 5 hydroxytryptamine (serotonin) cited in Table 10 7

**Phenylacetylglutamine and Phenylacetic Acid** Woolf [93] detected phenylacetic acid for which no satisfactory methods of measurement are available and also its principal conjugation product with glutamine Small amounts are said to be conjugated with glucuronic acid [12]

No definite quantitative statements can be made about the excretion

of the usual symptoms of untreated phenylketonuria [8 53] Woolf et al [51] described day to day variations in behavior correlated with plasma phenylalanine levels Phenylacetate administered in extremely large doses was without effect except to increase the characteristic odor of the urine A limited toxicity of this compound was noted many years ago in the course of experiments designed to test the limit of synthesis of glutamine with which this compound is conjugated Massive doses of phenylacetate several times the amount formed by phenylketonuric subjects were repeatedly taken by volunteer subjects with discomfort but with no truly alarming symptoms [108 109] Most of the other metabolites have been administered at one time or another without untoward effects

It has been suggested that the lower than normal level of serotonin in the blood of these patients which is presumably due to impaired synthesis from 5-hydroxytryptophan might contribute to the mental deficiency in phenylketonuric patients [90] The possibility of administering 5 hydroxytryptophan which can pass the blood brain barrier to make up for a deficiency was suggested

Pharmacologic studies of o-tyrosine the presumed precursor of o-hydroxyphenylacetic acid are of considerable interest o-Tyrosine passes the blood brain barrier and is decarboxylated to o-tyramine The latter is concentrated in the brain and the liver and its concentration can be still further elevated by iproniazid which inhibits its oxidation to o-hydroxyphenylacetic acid The effects of this on animals are marked central hyperactivity and sympathetic excitation [97] The precursors of this metabolite have not been found in phenylketonuric tissues but very small amounts could exert pharmacologic effects and could be missed by the usual analyses

## ORIGIN OF METABOLITES

Figure 10-7 indicates by double arrows the major metabolic pathways of phenylalanine tyrosine and tryptophan under normal conditions As a consequence of the block in the normal conversion of phenylalanine to tyrosine phenylalanine must detour through the other little used but not abnormal pathways as indicated Some metabolites of tyrosine and tryptophan are also diverted along unusual pathways

It has been suggested that there is inhibition of some of the steps in the normal metabolism of tryptophan and of tyrosine in phenylketonuria The evidence is indirect but there is indeed an accumulation of derivatives of the α keto acids of phenylalanine o-tyrosine tyrosine and tryptophan The keto acids are the parent substances which can give rise to the standard series of metabolites already listed the related pyruvic lactic acetic and conjugated acetyl form Knowledge of the individual enzyme reactions on which the metabolic map (Fig 10-7) is based is

alanine levels with the amount of phenylpyruvic acid excreted [20] and also with the amount of o-hydroxyphenylacetic acid excreted [91] Phenylalanine excretion is also proportional to its plasma level [10, 105] It is probably true for all of the metabolites that their excretion rate in the urine is proportional to plasma phenylalanine over certain ranges

The urinary concentrations of three compounds phenylpyruvic acid o-hydroxyphenylacetic acid and phenylalanine have been tested as convenient indicators of the plasma phenylalanine concentration during treatment of patients Phenylpyruvic acid excretion ceases at phenylalanine plasma levels below 0 6 μmoles per ml (10 mg per 100 ml) [106] or 0 90 μmoles per ml (15 mg per 100 ml) [5] The latter value is probably high o-Hydroxyphenylacetic acid excretion was detected at plasma phenylalanine levels above 0 48 or 0 67 μmoles per ml (8 or 11 mg per 100 ml) [106, 107] o-Hydroxyphenylacetic acid may therefore be slightly superior as a measure of plasma phenylalanine but at the critical lower levels excretion of phenylalanine itself may be superior to either acid [106]

*Quantitative Relations of Metabolites to Intelligence*

For the vast majority of patients there exists no correlation between intelligence and amounts of any of the metabolites measured But it must be remembered that chemical measurements have generally been made on phenylketonuric patients of all ages and not at the time when the mental damage was done

Tabulated plasma phenylalanine levels and I Q s for 18 patients [86] for another 18 patients [87] and for 34 patients [91] show that there is no relationship There was also no relation between I Q s of 8 patients and their cerebrospinal fluid levels of phenylalanine [86] Jervis [85] found that the higher plasma phenylpyruvic acid levels were associated with higher I Q 's and with better nutritional states in 34 patients He attributed the low levels of the severely defective patients to their chronic malnutrition 5 Hydroxytryptamine levels were independent of the I Q s of 9 patients [90]

Since no relation between intelligence as measured by the I Q test and plasma phenylalanine concentrations exists none would be expected with the urinary metabolites that are formed in proportion to the plasma phenylalanine This has been borne out in a number of studies [49 64 88 90, 91]

*Pharmacology of Metabolites*

Phenylketonuric patients under treatment with a low phenylalanine diet have been used to a limited extent to test the pharmacologic effects of the various metabolites Only phenylalanine (or phenylpyruvate) administration produced definite effects on these patients These were slight ataxia and deterioration of behavior and reappearance or worsening

described below Each step in the map has been numbered for convenience in discussion

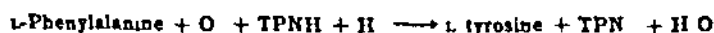
An alternative pathway of phenylalanine degradation which has not been investigated in phenylketonuria is that giving benzoic acid by a cleavage reaction [110] This should result in increased hippuric acid excretion Armstrong Chao Parker and Wall demonstrated this conversion in rats [111]

### *Phenylalanine Hydroxylase (A1)*

The original isotopic demonstration of the phenylalanine hydroxylase reaction was in rats Deuterium labeled phenylalanine was metabolized to labeled tyrosine isolated from the tissue proteins [112] The observation was then repeated in phenylketonuric patients with $C^{14}$ phenylalanine [113] Less than 10 per cent of the usual amount of tyrosine was formed from phenylalanine The small amount formed may be attributed to other unknown reactions or to residual activity of the genetically distorted enzyme protein Residual activity has not been detected in studies of the enzyme in phenylketonuric liver Direct studies of the enzyme from patients were first reported by Jervis [5] and later by others [114–116]

Phenylalanine hydroxylase assays in liver from normal controls and a total of seven phenylketonuric patients have proved that the enzyme is present in specimens from normal man but that there is no detectable activity in phenylketonuric liver The enzyme requires for activity a second protein fraction which is widely distributed in the body either reduced DPN or TPN and a new coenzyme related to folic acid (see below) All these cofactors are present in phenylketonuric liver Lack of phenylalanine hydroxylase activity is referable to a fault of the specific enzyme itself It has not yet been determined whether an altered protein is present in place of the normal enzyme protein or whether the genetic defect is a gene deletion

Phenylalanine hydroxylase occurs only in the liver It is found in the liver only after the biochemical differentiation following birth [117] Its reaction has the following stoichiometry


L-Phenylalanine + O + TPNH + H ⟶ L-tyrosine + TPN + H O

Oxygen is required for the reaction One atom is used in the *p*-hydroxylation and one is simultaneously reduced to water by the associated oxidation of the reduced pyridine nucleotide The reaction is therefore one of a newly recognized group of oxygenations in which gaseous oxygen is incorporated directly into the substrate A necessary coenzyme which has not been identified can be replaced by tetrahydrofolic acid although the latter does not function catalytically [118] The reaction is specific for L-phenylalanine It does not hydroxylate other aromatic compounds nor

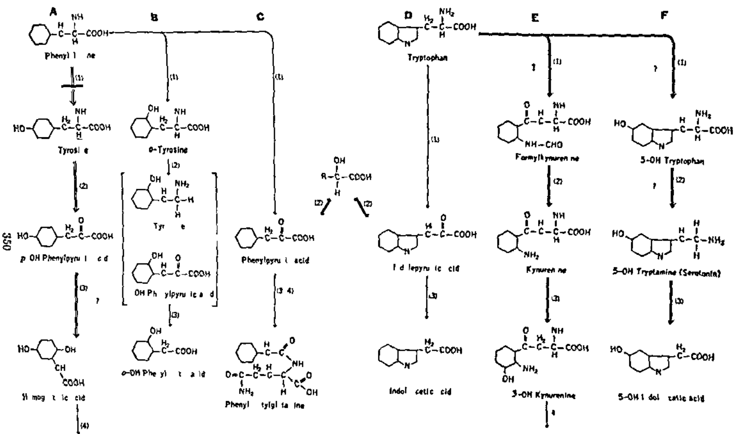



Fig 10 7 Map of the main normal metabolic routes of phenylalanine tyrosine and tryptophan (double arrows) the location of the real and apparent blocks in phenylketonuria and the alternate routes (single arrows) giving rise to the metabolites t a t t of the disease The individual reactions are described in the text

details are given in Chap 12 Alcaptonuria The final products are fumaric and acetoacetic acids which can be further oxidized in the citric acid cycle

*Conversion of Phenylalanine to o-Tyrosine o-Tyramine and o-Hydroxyphenylacetic Acid (B1 2 and 3)*

*o*-Hydroxyphenylacetic acid is formed from phenylalanine The former compound appears in the urine labeled with $C^{14}$ after administration of phenylalanine $C^{14}$ [*129*] and the amount excreted is proportional to the concentration of phenylalanine in the plasma [*91 107*] The two possible pathways of formation are shown in the figure It is most probable that *ortho*-hydroxylation occurs first to form *o*-tyrosine The enzyme has not been identified *o*-Tyrosine is excreted as *o* hydroxyphenylacetic acid [*130*] An alternate route through the pyruvate derivative was demonstrated by Flatow [*131*] Decarboxylation of *o* tyrosine to *o*-tyramine by guinea pig kidney and rat liver extracts was demonstrated by Blaschko [*132*] This occurs slightly less rapidly than decarboxylation of dihydroxyphenylalanine (dopa) Both reactions may be catalyzed by the dopa decarboxylase If so the suggestion will have to be modified that the latter enzyme is inhibited by metabolites in phenylketonuria and that epinephrine synthesis is decreased in this way [*133*] The oxidation of *o*-tyramine by amine oxidase was described by Randall [*134*] The pathway from *o*-tyrosine through *o*-tyramine to *o*-hydroxyphenylacetic acid was demonstrated in animals by Mitoma et al [*97*] At present it can only be said that both routes are possible

*Phenylalanine Transaminase (C1)*

The suggestion that phenylpyruvate in phenylketonuria comes from transamination of phenylalanine was first made by Utena and Saito [*?6*] They administered glutamic acid to their patient and noted that the amount of phenylpyruvic acid excreted was decreased This could be explained by reversal of the phenylalanine transaminase reaction



**Phenylalanine + α ketoglutarate → phenylpyruvate + glutamate**

Meister et al [*103*] have also reported inhibition of phenylpyruvate excretion after administration of glutamic acid or glutamine to two phenylketonuric patient These observations also implicated transamination in the origin of phenylpyruvate

Like the two tyrosine transaminases there are two phenylalanine transaminases which require α ketoglutarate and pyruvate respectively Both require pyridoxyl phosphate as the coenzyme and both are in highest concentration in liver [*1?5 1? 13?*] The observation that the phenylalanine transaminase appears only after birth in rat liver [*13?*]

does it hydroxylate in the *ortho* or *meta* positions. A brief review of this and related enzymes can be found elsewhere [119].

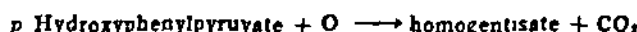
A nonenzymatic system of ferrous ion, ascorbic acid, a chelating agent and oxygen will hydroxylate phenylalanine [120]. It has been suggested that *o*-tyrosine, as well as a little tyrosine, might be formed in this way in phenylketonuric patients [121, 122], but there is no proof that such a nonenzymatic reaction plays a role in metabolism.

*Tyrosine Transaminase* (12)

L-Tyrosine is first converted to its keto acid in the course of its oxidation by transamination [123]. There are two specific tyrosine transaminases, one reacting with α-ketoglutarate and one with pyruvate as acceptors [124]. Both require pyridoxal phosphate. The former enzyme has been partially purified [125]. It does not react with other aromatic amino acids. The liver enzyme tyrosine α-ketoglutarate transaminase is induced to greatly increased levels within a few hours by administration of the substrate or hydrocortisone [126]. Small amounts of the enzyme also occur in the kidney.

*p-Hydroxyphenylpyruvate Oxidase* (13)

This unusual enzyme catalyzes the first oxidative step in the main metabolism of tyrosine. It occurs in liver and in smaller amounts in kidney. The reaction is also an oxygenation and forms homogentisate without intermediates (2,5-dihydroxyphenylpyruvate is not formed). Ring hydroxylation and migration and oxidative decarboxylation of the side chain apparently occur together.


$$p\text{-Hydroxyphenylpyruvate} + O \longrightarrow \text{homogentisate} + CO_2$$

The enzyme has been partially purified and extensively studied [127, 128]. A point of considerable interest is the inactivity of the reaction in scorbutic animals. These animals excrete *p*-hydroxyphenylpyruvate when fed tyrosine. Ascorbic acid or certain other compounds are required to prevent reaction product inactivation of the enzyme in vitro.

The enzyme is completely inhibited by 0.002 *M* phenylpyruvate. It is the enzyme most sensitive to inhibition by the metabolites of phenylketonuria that has been described [128]. Nevertheless, *p*-hydroxyphenylpyruvate is not excreted in large amounts in phenylketonuria, even after large doses of tyrosine. The concentration of phenylpyruvate in plasma in phenylketonuria is one fiftieth of the concentration which inhibits *p*-hydroxyphenylpyruvate oxidase.

*Homogentisate Oxidase* (14)

The enzymes responsible for homogentisate oxidation and the subsequent reactions of tyrosine degradation are all known [123]. Further

as coenzyme The activity in liver of the more active enzyme which requires $\alpha$-ketoglutarate is controlled by hydrocortisone [135]

The enzymatic mechanisms for the further conversion of the indole compounds to indoleacetic acid and its conjugates are unknown

*Tryptophan Oxidation (I F)*

Ring oxidation of tryptophan takes place either by opening of the ring to form kynurenine and ultimately nicotinic acid [141] (E4) or by introduction of the 5 hydroxy group in the serotonin series [142] Only the 5 hydroxylation of tryptophan itself remains to be described in enzymatic terms (I 1) The largest amount of tryptophan is oxidized by opening of the indole ring to form formylkynurenine [143] (F1) This enzyme now called tryptophan pyrrolase is another which catalyzes an oxygenation reaction It is not known to be inhibited by any of the metabolites of phenylketonuria

5 Hydroxytryptophan decarboxylase (F2) is a specific pyridoxal requiring enzyme found in a number of tissues including liver and brain It has been suggested that the low level of serotonin in the blood of phenylketonuric patients arises from depressed activity of this enzyme [144] Davison and Sandler [145] found that the enzyme was inhibited 50 per cent by 0 02M phenylpyruvic acid and somewhat less by other metabolites of phenylketonuria This concentration is 500 times the amount of phenylpyruvic acid found in the blood in phenylketonuria (0 043 $\mu$moles per ml)

*Summary of the Origin of Metabolites*

*The inactivity of the phenylalanine hydroxylase is firmly established* as the primary defect in phenylketonuria The minor metabolic pathways employed by phenylalanine in detouring around this metabolic block are well outlined The various enzymatic reactions are known with the exception of the reaction leading to the o-hydroxyphenyl series and the reactions for interconverting a few of the minor metabolites Uncertainty exists only about the origin of defects in pathways unrelated to phenylalanine Inhibition by phenylpyruvic acid or its derivatives of the degradation of tryptophan and tyrosine and the formation of serotonin may occur but the evidence is indirect In addition to the enzyme inhibitions cited above Bickis Kennedy and Quastel [146] have demonstrated inhibition by phenylalanine both of tyrosine degradation and of tyrosine incorporation into proteins by tissue slices

Inhibitions of these sorts may be possible explanation of some of the abnormalities which are therapeutically reversible in phenylketonuria but so also may be adaptive changes in the metabolic processes brought on by the unusual chemical milieu of the tissues For example a general increase in the rate of transamination of tyrosine and tryptophan with

may be relevant to the finding that excretion of phenylpyruvate may be delayed until 1 month after birth in phenylketonuria [137]

*Lactic Dehydrogenase (C2)*

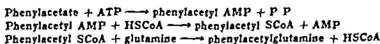
Lactic dehydrogenase catalyzes the reversible reduction of α keto acids by DPNH to their lactic acid derivatives It is undoubtedly responsible for the appearance of the lactic derivatives of the α keto metabolites in phenylketonuria The specificity of this enzyme is low Meister [138] demonstrated that it reacts with phenylpyruvate p-hydroxyphenylpyruvate and indolepyruvate at about one-one hundredth the rate of its reaction with pyruvic acid In view of the large amounts of this enzyme in the body these relatively slow rates are adequate to account for the quantities of lactic acid derivatives which are formed

*Phenylacetate Formation (C3)*

The mechanisms for the conversion of aromatic α-keto acids to the acetic acid derivatives are not known The reactions are analogous in type to the oxidative decarboxylations of pyruvate to acetate or of α-ketoglutarate to succinate

*Phenylacetylglutamine Synthesis (C4)*

This conjugation product is formed only by man and the higher apes The enzymatic synthesis is similar to those involved in protein formation Moldave and Meister [139] showed that the synthesis occurs by activation of phenylacetate with ATP then transfer of the phenylacetyl group, first to CoA and then to glutamine



Phenylacetate + ATP ⟶ phenylacetyl AMP + P P
Phenylacetyl AMP + HSCoA ⟶ phenylacetyl SCoA + AMP
Phenylacetyl SCoA + glutamine ⟶ phenylacetylglutamine + HSCoA

Phenylacetylglutamine is the only one of the characteristic metabolites of phenylketonuria whose formation has been measured in the tissues of a phenylketonuric patient Meister [140] found that a liver biopsy sample from a phenylketonuric patient formed phenylacetylglutamine approximately five times faster than the rate observed in liver preparations from patients without phenylketonuria This suggests that the enzyme is *adaptive in nature and indicates one way in which the body* may adjust to handle unusual amounts of metabolites by alternate metabolic routes

*Tryptophan Transaminases (D1)*

Like the other transaminations mentioned this reaction is also catalyzed by two specific enzymes one using α ketoglutarate and the other pyruvate as the acceptors The enzymes require pyridoxal phosphate

acid level in plasma (Table 10-7) is one hundredth this concentration (0 013 μmoles per ml or 0 000013 M) the possibility of some in vivo inhibition of this enzyme is remote If this enzyme is also involved in the formation of o-hydroxyphenylacetic acid by decarboxylation of o-tyrosine it might also be competitively inhibited

*β-Globulins of Serum and Hemoglobin Synthesis*

Electrophoretic studies of serum in 17 cases of phenylketonuria have revealed multiple abnormal sharp high peaks in the β globulin fraction in 8 cases [87] This fraction contains the β lipoproteins Similar findings were encountered in one case of active hepatitis The abnormal components disappeared in those patients treated with a low phenylalanine diet and reappeared when phenylalanine was restored to the diet Thus the high phenylalanine concentration is the factor responsible for the abnormal β globulin components

The possibility of an error in the synthesis of one protein hemoglobin in the presence of the high concentration of phenylalanine was excluded by the precise analysis by Allen and Schroeder [103] The incorporation of phenylalanine into hemoglobin by a phenylketonuric subject was correct to within less than one residue per molecule Thus protein synthesis may be remarkably accurate even in abnormal conditions but it is possible that other proteins in phenylketonuria might be formed with an excess phenylalanine content

*Cerebral Oxygen Consumption*

Oxygen arteriovenous differences in the cerebral circulation were lower in nine phenylketonuric patients than in other comparable mentally defective patients [164 165] Since blood flow measurements were not made it is not known whether cerebral oxygen uptake was actually decreased The oxygen uptake per gram may still be normal because of the reduced brain weight of these patients

*Altered Pattern of Plasma Amino Acids*

The abnormalities of the biochemical milieu in which the cells of phenylketonuric patients must live and grow have not been defined as well as might be Knowledge of the nutritional requirements at the cellular level is inadequate to predict the consequences of these abnormalities One example of the potentially harmful alterations of the cellular environment is the distorted pattern of free amino acids The α-amino nitrogen of the phenylalanine found in this disease is about half of the normal total α amino nitrogen of plasma yet the total α-amino nitrogen in phenylketonuric plasma is not substantially higher than normal [ ?] Obviously the concentrations of other amino acids must be depressed

formation of excess proportions of their α keto acids would equally well account for the observed abnormalities in metabolism of these compounds The question can be settled only by measurements of the reactions in question under physiologic conditions in the phenylketonuric patient

## OTHER BIOCHEMICAL ABNORMALITIES

### *The Conversion of Tyrosine to Melanin Pigment*

The decreased pigmentation in phenylketonuria is accounted for by competitive inhibition of phenylalanine on the tyrosinase system Daneis and Balis [147] first demonstrated competition between phenylalanine and tyrosine with a mushroom tyrosinase system Miyamoto and Fitzpatrick [148] confirmed this with mammalian tyrosinase An unusually clear demonstration of the phenomenon of competitive inhibition under physiologic conditions is provided by the darkening of new grown hair of phenylketonuric patients when either tyrosine intake is increased [149] or phenylalanine intake is lowered [5]

### *Epinephrine Tolerance*

There are suggestions that phenylketonuric patients are deficient in epinephrine A test involving serial blood pressure measurements following injections of Mecholyl and of epinephrine has been used to determine the autonomic reactivity of psychiatric patients [150] Cawte [151] found that two phenylketonuric patients reacted like the controls to Mecholyl but had an increased hypertensive response to epinephrine. This finding was later supported [152] by studies on 7 phenylketonuric patients Only 7 of 30 phenylketonuric subjects were able to give regularly the necessary cooperation Those selected had on the average half again as high a rise in blood pressure as a group of 9 nonphenylketonuric mentally defective control patients and they required a longer period for return to basal values after epinephrine injection The difference between the two groups is statistically significant Cawte related this responsiveness of phenylketonuric patients to their tense reactive attitude Another explanation is found in Cannon s rule that a neuroreceptor deprived of its usual stimulus becomes more sensitive Plasma concentrations of epinephrine were actually found to be lower than normal in 13 phenylketonuric children but they were also low in patients with other kinds of mental defect [96] Measurement of the epinephrine in adrenal glands from one patient showed a normal or high value [121]

The inhibition of dopa decarboxylase by metabolites of phenylketonuria has been suggested as an explanation of the low epinephrine plasma value (or increased sensitivity to epinephrine) [77] Phenylpyruvate and lactate are the most effective inhibitors They have a pronounced effect in concentrations as low as 0 003 M Since the average phenylpyruvic

acid level in plasma (Table 10 7) is one hundredth this concentration (0 043 μmoles per ml or 0 000043 M) the possibility of some in vivo inhibition of this enzyme is remote If this enzyme is also involved in the formation of o-hydroxyphenylacetic acid by decarboxylation of o tyrosine it might also be competitively inhibited

*β-Globulins of Serum and Hemoglobin Synthesis*

Electrophoretic studies of serum in 17 cases of phenylketonuria have revealed multiple abnormal sharp high peaks in the β-globulin fraction in 8 cases [87] This fraction contains the β lipoproteins Similar findings were encountered in one case of active hepatitis The abnormal components disappeared in those patients treated with a low phenylalanine diet and reappeared when phenylalanine was restored to the diet Thus the high phenylalanine concentration is the factor responsible for the abnormal β globulin components

The possibility of an error in the synthesis of one protein hemoglobin in the presence of the high concentration of phenylalanine was excluded by the precise analysis by Allen and Schroeder [153] The incorporation of phenylalanine into hemoglobin by a phenylketonuric subject was correct to within less than one residue per molecule Thus protein synthesis may be remarkably accurate even in abnormal conditions but it is possible that other proteins in phenylketonuria might be formed with an excess phenylalanine content

*Cerebral Oxygen Consumption*

Oxygen arteriovenous differences in the cerebral circulation were lower in nine phenylketonuric patients than in other comparable mentally defective patients [154 155] Since blood flow measurements were not made it is not known whether cerebral oxygen uptake was actually decreased The oxygen uptake per gram may still be normal because of the reduced brain weight of these patients

*Altered Pattern of Plasma Amino Acids*

The abnormalities of the biochemical milieu in which the cells of phenylketonuric patients must live and grow have not been defined as well as might be Knowledge of the nutritional requirements at the cellular level is inadequate to predict the consequences of these abnormalities One example of the potentially harmful alterations of the cellular environment is the distorted pattern of free amino acids The α-amino nitrogen of the phenylalanine found in this disease is about half of the normal total α amino nitrogen of plasma yet the total α amino nitrogen in phenylketonuric plasma is not substantially higher than normal [52] Obviously the concentrations of other amino acids must be depressed

Only one analysis of plasma amino acids in phenylketonuria has apparently been done This is referred to briefly by Stein and Moore [57] The values of the amino acids in this study (personal communication from W H Stein and S Moore) appear in Fig 10 8 and are compared with the average plasma values from five fasting adult males obtained by

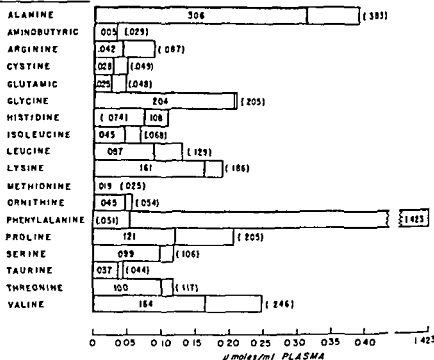



Fig 10-8 Amounts of some of the free plasma amino acids in a 33 year-old male phenylketonuric patient (I Q 44) (shaded columns) superimposed on the mean values for five normal adult males (recalculated and drawn from data of Stein and Moore [57] and personal communications) It appears that the high level of phenylalanine may depress the levels of most other amino acids with maintenance of a normal value for the total α amino nitrogen

column chromatography by the same authors The high phenylalanine evidently caused a depression of the concentration of most other amino acids Glycine and histidine are unique in being present at a normal and an increased level respectively Of the undetermined amino acids only glutamine normally occurs in significant amounts and it also may be changed in phenylketonuria

### Renal Clearance

The renal clearance of phenylalanine in both the dog [104] and man [105] has been determined Like the majority of amino acids phenyl

alanine is well reabsorbed Clearance was about 1 to 2 ml per min At normal blood concentrations only about 1 per cent of the filtered phenyl alanine appears in the urine but as much as 15 per cent of that filtered appears in the urine when the concentration in the serum is raised tenfold A $T_m$ (tubular secretion max ) cannot be reached with the plasma phenyl alanine levels obtainable in normal persons but it would appear from the studies above that the $T_m$ would be exceeded at slightly higher levels than those studied No measurements of renal clearance have been reported in phenylketonuric patients Here the blood levels obtainable would permit the direct measurement of the $T_m$

*Experimental Phenylketonuria*

An experimental approach to this disease was opened by Auerbach et al [156 157] who found that phenylalanine hydroxylase synthesis is repressed by administration of tyrosine the product of the reaction This phenomenon which is the reverse of substrate induction of an enzyme had not previously been known in animals although it had been observed in microorganisms Phenylalanine hydroxylase activity was reduced to one twentieth of its usual value by maintaining young rats on a 5 per cent tyrosine diet The addition of extra phenylalanine to this diet did not alter the enzyme activity since the enzyme is not adaptively increased by its substrate The excretion of phenylpyruvic acid by these animals was high but this may have been because DL-phenylalanine was given However the plasma level of L-phenylalanine was lightly raised and other tests suggested that the treated animals showed some retardation of learning ability These experiments are an ingenious duplication by environmental influences of the metabolic picture produced by an hereditary enzyme defect

## GENETICS

Familial cases appeared among the first reported patients with phenyl ketonuria and perhaps this was re pon ible for the initial recognition of the di ease There were three sib pairs among Følling s first 10 patients It is reported [158] that the mother of two of the original patients struck by their similarities and particularly by their malodorous urines insisted that special biochemical ob ervations be made

The genetics of phenylketonuria is now precisely known Jervis [12] had this to say of the factors facilitating the genetic study 1) the iden tification of this condition is simple and exact being made by a chemical test 2) the character segregates sharply affected individuals being entirely different biochemically from nonaffected ones 3) the disease fulfills the requirement of being a unit in a biological (biochemical) sen e It should be added that the disea e is rare for this permits some

Only one analysis of plasma amino acids [illegible]

[illegible]

AMINO ACIDS

[illegible]

0 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 1.40

Fig. 128. [illegible] IQ [illegible]

[illegible] Glycine and histidine [illegible]

*Blood Chemistry*

[illegible]

alanine is well reabsorbed Clearance was about 1 to 2 ml per min At normal blood concentrations only about 1 per cent of the filtered phenyl alanine appears in the urine but as much as 15 per cent of that filtered appears in the urine when the concentration in the serum is raised tenfold A $T_m$ (tubular secretion max ) cannot be reached with the plasma phenyl alanine levels obtainable in normal persons but it would appear from the studies above that the $T_m$ would be exceeded at slightly higher levels than those studied No measurements of renal clearance have been re ported in phenylketonuric patients Here the blood levels obtainable would permit the direct measurement of the $T_m$

*Experimental Phenylketonuria*

An experimental approach to this disease was opened by Auerbach et al [*156 157*] who found that phenylalanine hydroxylase synthesis is repressed by administration of tyrosine the product of the reaction This phenomenon which is the reverse of substrate induction of an enzyme had not previously been known in animals although it had been observed in microorganisms Phenylalanine hydroxylase activity was reduced to one twentieth of its usual value by maintaining young rats on a 5 per cent tyrosine diet The addition of extra phenylalanine to this diet did not alter the enzyme activity since the enzyme is not adaptively increased by its substrate The excretion of phenylpyruvic acid by these animals was high but this may have been because DL-phenylalanine was given However the plasma level of L-phenylalanine was slightly raised and other tests suggested that the treated animals showed some retardation of learning ability These experiments are an ingenious duplication by environmental influences of the metabolic picture produced by an hereditary enzyme defect

## GENETICS

Familial ca es appeared among the first reported patients with phenyl ketonuria and perhaps this was re ponsible for the initial recognition of the disease There were three sib pairs among Følling s first 10 patients It is reported [*158*] that the mother of two of the original patients struck by their similarities and particularly by their malodorous urines insisted that special biochemical observations be made

The genetics of phenylketonuria is now preci ely known Jervis [*12*] had this to say of the factors facilitating the genetic study 1) the iden tification of this condition is simple and exact being made by a chemical test 2) the character egregates sharply affected individuals being entirely different biochemically from nonaffected ones 3) the disease fulfills the requirement of being a unit in a biological (biochemical) sen e It should be added that the disease is rare for this permits some

simplified assumptions in handling the distribution data. These rare cases are largely concentrated into institutions where they can be found, observed, and reported in sizable groups. Consequently, more cases of phenylketonuria are known than of any other comparably rare disease.

## MODE OF INHERITANCE

The first large-scale genetic analysis by Jervis [9] comprised 213 patients and indicated that the disease is transmitted by a single autosomal

TABLE 10-9 FREQUENCY OF PHENYLKETONURIC OFFSPRING IN VARIOUS MATINGS

| *Marital partners* | | | | *Theoretic frequency of affected children if both partners were carriers* | *Chances of affected children from such a mating* |
|---|---|---|---|---|---|
| *A* | | *B* | | | |
| *Carrier status* | *Chances of carrying gene* | *Carrier status* | *Chances of carrying gene* | | |
| Unknown | 1/80 | Unknown | 1/80 | 1/4 | 1/25 600 |
| Unknown | 1/80 | Normal sibling of phenylketonuric | 2/3 | 1/4 | 1/480 |
| Unknown | 1/80 | Parent of phenylketonuric | 1 | 1/4 | 1/320 |
| Unknown | 1/80 | Phenylketonuric | 1 | 1/2 | 1/160 |
| Normal sibling of phenylketonuric | 2/3 | Normal sibling of phenylketonuric | 2/3 | 1/4 | 1/9 |
| Normal sibling of phenylketonuric | 2/3 | Parent of phenylketonuric | 1 | 1/4 | 1/6 |
| Normal sibling of phenylketonuric | 2/3 | Phenylketonuric | 1 | 1/2 | 1/3 |
| Parent of phenylketonuric | 1 | Parent of phenylketonuric | 1 | 1/4 | 1/4 |
| Parent of phenylketonuric | 1 | Phenylketonuric | 1 | 1/2 | 1/2 |
| Phenylketonuric | 1 | Phenylketonuric | 1 | 1 | 1 |

Calculated on prevalence rate of disease as approximately 4/100 000 as suggested by Jervis.

SOURCE: S. W. Wright et al. [15].

recessive gene. His definitive review [12] included data from 146 personally observed families, 46 families of Munro [17], 22 families of Følling, Mohr, and Ruud [10], and 52 other families reported in the world literature up to 1954. In the 266 families were 1,094 siblings, 433 (39.6 per cent) with phenylketonuria. On the hypothesis of a single autosomal recessive gene, 25 per cent would be affected. Corrections for the method of ascertainment by the methods of Weinberg and Lenz, which allowed for the uncounted families with only normal children, gave corrected

percentages with standard errors of affected children as 27 37 ± 2 57 (sib method) 22 38 ± 2 66 (proband method) and within one third of the standard error of the hypothetic 25 per cent by the Lenz a priori method There was no significant difference in the sex incidence The evidence for autosomal recessive transmission was further substantiated by the occurrence of parental consanguinity in 8 33 per cent of 206 of these

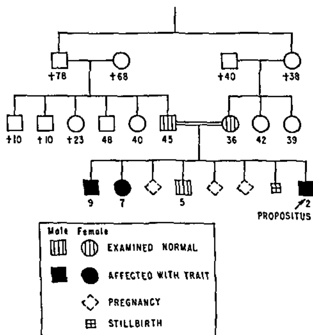



Fig 10-1 Pedigree of Japanese family with phenylketonuria illustrating parental consanguinity parental normality and multiple siblings affected [27] Age in years and deaths (+) are indicated Other symbols are standard

families from the United States England and Norway This is the incidence expected for a recessive disease occurring once in 25 000 times in a population that has approximately 1 per cent cousin marriages The precision with which the inheritance of phenylketonuria conforms to expectations lends considerable weight to the simple mathematical calculation of the risk of this disease in different types of matings as shown in Table 10-9

In the vast majority of families the parents are normal are often related and rarely have affected relatives A typical pedigree is shown in Fig 10-9 It is probable that in the few twin pairs reported the monozygotic pairs are concordant while the dizygotic pairs are either concordant or discordant as expected (Table 10-10) Sixty five half sibs of affected

simplified assumptions in handling the distribution data These rare cases are largely concentrated into institutions where they can be found observed and reported in sizable groups Consequently more cases of phenylketonuria are known than of any other comparably rare disease

## MODE OF INHERITANCE

The first large scale genetic analysis by Jervis [3] comprised 213 patients and indicated that the disease is transmitted by a single autosomal

TABLE 10-9 FREQUENCY OF PHENYLKETONURIC OFFSPRING IN VARIOUS MATINGS

| *Marital partners* | | | | *Theoretic frequency of affected children if both partners were carriers* | *Chances of affected children from such a mating* |
|---|---|---|---|---|---|
| *A* | | *B* | | | |
| *Carrier status* | *Chances of carrying gene* | *Carrier status* | *Chances of carrying gene* | | |
| Unknown | 1 80 | Unknown | 1 80 | 1 4 | 1 25 600 |
| Unknown | 1 80 | Normal sibling of phenylketonuric | 2 3 | 1 4 | 1 480 |
| Unknown | 1 80 | Parent of phenylketonuric | 1 | 1 4 | 1 320 |
| Unknown | 1 80 | Phenylketonuric | 1 | 1 2 | 1 160 |
| Normal sibling of phenylketonuric | 2 3 | Normal sibling of phenylketonuric | 2 3 | 1 4 | 1 9 |
| Normal sibling of phenylketonuric | 2 3 | Parent of phenylketonuric | 1 | 1 4 | 1 6 |
| Normal sibling of phenylketonuric | 2 3 | Phenylketonuric | 1 | 1 2 | 1 3 |
| Parent of phenylketonuric | 1 | Parent of phenylketonuric | 1 | 1 4 | 1 4 |
| Parent of phenylketonuric | 1 | Phenylketonuric | 1 | 1 2 | 1 2 |
| Phenylketonuric | 1 | Phenylketonuric | 1 | 1 | 1 |

Calculated on prevalence rate of disease as approximately 4 100 000 as suggested by Jervis

SOURCE S W Wright et al [15]

recessive gene His definitive review [12] included data from 146 personally observed families 46 families of Munro [17] 22 families of Følling Mohr, and Ruud [10] and 52 other families reported in the world literature up to 1954 In the 266 families were 1 094 siblings 433 (39 6 per cent) with phenylketonuria *On the hypothesis of a single autosomal recessive gene* 25 per cent would be affected Corrections for the method of ascertainment by the methods of Weinberg and Lenz which allowed for the uncounted families with only normal children gave corrected

of members of affected families have been recorded in a search for linkage of the blood groups with the gene for phenylketonuria [17 38 160] If such linkage exists at all it is loose and not close enough to identify the heterozygotes Psychoses were suggested as more frequent among parents and other relatives of phenylketonuric patients but statistical studies have not confirmed (or denied) this [3 17] The possibility deserves further study with the aid of the chemical tests recently developed for identifying heterozygotes

*Chemical Identification of Heterozygotes of Phenylketonuria*

With Jervis direct proof that phenylalanine hydroxylase activity is missing from the livers of patients with phenylketonuria [6] and his final proof that this is a recessive disease [12] it was considered possible that the heterozygous carriers with one defective gene would have less than the normal complement of phenylalanine hydroxylase as suggested by C G King [161] during the discussion following the presentation of Jervis classical review Følling and Closs [162] hinted that there might be increased phenylalanine in the urine of heterozygotes Waelsch could find no abnormality in phenylalanine level in the blood in 10 pairs of parents [163]

This question was reinvestigated by Hsia Driscoll Troll and Knox [9] who gave standard doses of L-phenylalanine and followed the plasma concentration of phenylalanine by a specific enzymatic method Parents of known phenylketonuric patients were the heterozygotes Their phenylalanine concentrations were higher and more sustained after the given dose than those of control individuals The results are shown graphically in Fig 10 10 The data from this study were published in detail by Hsia and Paine [164] The same distinction between 10 heterozygotes and 10 controls was confirmed by Berry Sutherland and Guest [85] They measured serum phenylalanine by a chromatographic method which gave about 30 per cent lower values than the specific enzymatic method but it revealed the same relative difference between known heterozygotes and controls (Fig 10-11)

Further investigation disclosed that more detailed measurements could detect a subtle abnormality even under basal conditions Knox and Messinger [60] refined the specific enzymatic method to determine more precisely the normal levels of phenylalanine and were able to show that a group of heterozygotes had a significantly higher mean fasting level than a control group (Fig 10 12) There was the same degree of separation between the groups by this test as by the tolerance test The phenylalanine concentration of the heterozygote group was about 1 5 to 2 5 times the normal both in the fasting state and after the loading dose

The tolerance test therefore did not significantly enhance a latent abnormality nor did it reveal a deficient metabolic reserve since a

individuals were all normal as expected except for two whose father had married two sisters and produced a phenylketonuric offspring in each union [12]

Records of matings of affected individuals to normal individuals exist (Table 10 11) The phenylketonuric offspring in some of these matings

TABLE 10-10 PHENYLKETONURIA IN TWINS

| Reference | Zygosity | Phenylketonuria | Normal |
|---|---|---|---|
| [3] Jervis 1939 Cases 185 186 | Monozygous | 2 | |
| [3] Jervis 1939 Cases 21 22 | Monozygous (?) | 2 | |
| [15] Wright and Tarjan 1957 | Monozygous | 2 | |
| [3] Jervis 1939 Cases 158 159 | Dizygous | 2 | |
| [3] Jervis 1939 Case 1 | Dizygous | 1 | 1 |
| [159] Thompson 1957 | Not determined both males | 1 | 1 |
| [51] Woolf et al 1958 | Dizygous | 1 | 1 |
| [10] Følling Mohr and Ruud 1944 Case 11 | Dizygous (?) females | 1 | 1 (probably) |

TABLE 10-11 MATINGS OF PHENYLKETONURIC INDIVIDUALS

| Reference | Phenylketonuric parent | Other parent | Offspring |
|---|---|---|---|
| [12] | Female(?) | (Illegitimate union) | 1 normal |
| [21] | Female low grade | (Illegitimate union) | 1 probably normal idiot |
| [19] | High grade married female | Normal | 2 normal |
| [3] | Female imbecile Case 24 | Normal (heterozygote ?) | 2 normal<br>2 phenylketonuric |
| [93] | High grade married male | Normal (heterozygote ?) | 1 normal<br>1 phenylketonuric |

can be adequately explained by the heterozygosity of the apparently normal spouses Births of normal children from phenylketonuric mothers establish the fact that no intrauterine damage is done to the fetus by the metabolic abnormalities of the phenylketonuric mother The corollary that a phenylketonuric fetus would not harm himself by his own metabolites during intrauterine life is the basis for the view that phenotypic defects of the phenylketonuric child develop after birth

## HETEROZYGOTE CHARACTERISTICS

The concept of the recessivity of phenylketonuria is derived from failure to recognize any constant abnormality of the heterozygous carriers of the gene Chemical and intelligence test results of parents have been uniformly negative Data on ABO agglutinogens in the blood

PLASMA PHENYLALANINE (μ mols/ml)

1 2
1 1
1 0
0 9
0 8
0 7
0 6
0 5
0 4
0 3
0 2
0 1
0

PARENTS
CONTROLS

0 1 2 3 4 5
HOURS AFTER TEST DOSE

Fig 10-11 Tolerance curves following phenylalanine administration to 10 controls and 10 parents of phenylketonuric patients expressed in the same way as the results in Fig 10-10 (*Drawn from results of H Berry et al [8 ]*)

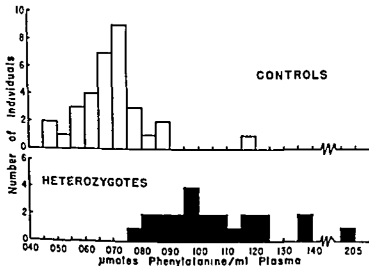



Fig 10-1 Distributions of plasma phenylalanine levels in 33 control and 23 heterozygous individuals [ 0]

similar degree of abnormality was present even under basal conditions Only a reduced capacity of heterozygotes to metabolize phenylalanine has been demonstrated but the phenylalanine hydroxylase reaction is the one deficient in heterozygotes because less tyrosine is formed Jervis [16a] has used the rise in tyrosine in plasma after a dose of phenylalanine as a test of heterozygosity This test is less practical since doses of the order of 25 gm are needed to show that the controls clearly form more tyrosine than do heterozygotes

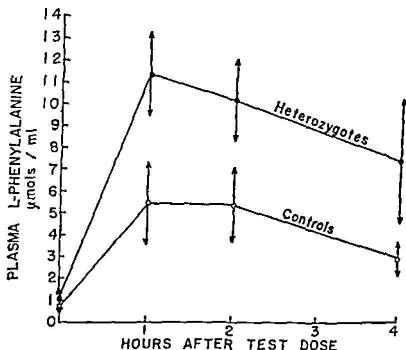



Fig 10-10 Plasma L-phenylalanine levels in normal (-o-) and heterozygous (-•-) persons following oral doses of 0 1 gm L-phenylalanine per kilogram body weight Each line represents the mean value from 19 persons The length of the arrows represents one standard deviation above and below the mean [58]

It has been suggested that the altered phenylalanine hydroxylase in phenylketonuria introduces an *ortho-* instead of a *para* hydroxyl group into phenylalanine and in this way gives rise to a qualitatively new (abnormal) o hydroxyphenyl series of metabolites [130] Berry et al [8a] found in the course of their tolerance test study that the heterozygote type of blood curve was almost invariably associated with urinary excretion of *o*-hydroxyphenylacetic acid and that no other consistent urinary product was found either in normal or heterozygous individuals Cullen and Knox [107] found that this substance is excreted by everyone when the plasma level of phenylalanine is sufficiently high This excretion therefore offers no improvement over the usual tolerance test

PARENTS
CONTROLS
PLASMA PHENYLALANINE (μmols/ml)
1 2, 1 1, 1 0, 0 9, 0 8, 0 7, 0 6, 0 5, 0 4, 0 3, 0 2, 0 1, 0
0 1 2 3 4 5
HOURS AFTER TEST DOSE

Fig 10-11 Tolerance curves following phenylalanine administration to 10 controls and 10 parents of phenylketonuric patients expressed in the same way as the result in Fig 10-10 (*Drawn from results of H Berry et al* [9])

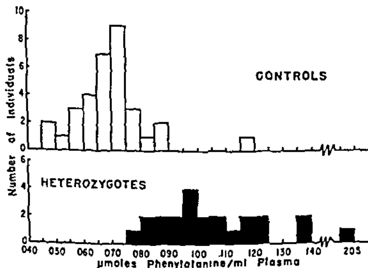



Fig 10-12 Distributions of plasma phenylalanine levels in 33 controls and 23 heterozygous individuals [10]

*Genetic Analysis Using the Chemical Test for Heterozygosity*

It would be of great interest and practicality if the genotype of a single individual could be determined from his metabolic phenotype The overlap between normal persons and heterozygotes in both tests described above was about 15 per cent A total of 12 apparently normal siblings of phenylketonuric patients was tested in the two studies Six were judged to be normal and six to be heterozygotes (expected 8 4) Also in each study one control fell well into the heterozygote range Such a frequency of true heterozygotes in the normal population (2 in 29) would be much higher than the calculated incidence of about 1 in 83 Obvious reasons such as liver disease which might also elevate phenylalanine concentrations, were excluded in these studies It is probable that other unidentified factors may alter phenylalanine levels The assignment of a person to one or the other genotype is uncertain with only this kind of information

*Incidence of Mental Disease in Heterozygotes*

This question has not been resolved in part because of the paucity of known heterozygotes available for study and because the incidence of unidentified phenylketonuric heterozygotes in the population (at least 1 per cent) is roughly comparable to the incidence of mental disease Heterozygotes could make up a large fraction of mental patients, but a very high susceptibility has doubtless not been overlooked

Følling s original report [1] stated that an aunt of one patient and a great aunt of another had dementia praecox He subsequently noted the incidence of psychoses in later life among relatives of the phenylketonuric patients but doubted that it was higher than was expected in the population as a whole [10] Penrose was primarily responsible for the further interest in this question He [160] reported the pedigree of a large family containing four aments at least one of whom was phenylketonuric There were 61 other individuals in the family and of these 6 showed definite signs of insanity of which the mean age of onset was about 50 years All of the 6 were carriers of the abnormal gene or at least there was a high probability that they were

The first controlled study of the incidence of mental disease in phenylketonuric families was that of Munro [17] Comparison with the incidence in 653 control families of imbecile and idiot patients showed that the incidence of psychoses in the phenylketonuric families was more than twice expectation but barely significant statistically The incidence of psychoses in the 47 phenylketonuric families increased with the age of the members the older the relatives the greater the chance of mental disease The most striking comparison was between family members

over age 45 where there was twice the expected incidence of mental disease in the phenylketonuric families

Thompson [159] described the relatives of 8 phenylketonuric patients and of 8 low grade mental defectives without phenylketonuria Mental deficiency or mental disorder of a serious nature was not found in the close relatives of the control patients but mental deficiency or mental disorder was found in each of the phenylketonuric families Omitting mental deficiency 13 relatives in 6 of the 8 phenylketonuric families had some kind of mild or severe mental disorder Three relatives in 2 of 8 control families had mild disorder Just over 100 relatives in each group were considered Serious disorders in the phenylketonuric families appeared in male relatives much more frequently than in female (1 female and 7 males) and except for 1 who first became psychotic at age 60 years all the disturbed persons had their mental disability throughout life

If the observed instances of mental disease are referred only to that fraction of the relatives who might be expected to be heterozygotes (e g all parents one half of the grandparents etc ) the corrected incidence of mental disease in heterozygotes in the families so far studied is of the order of 20 per cent This of course is a maximal incidence and one that is valid only *if* heterozygotes do have an increased incidence of mental disease Because mental disease and heterozygosity for phenylketonuria have similar incidences heterozygosity could account for as much as one fifth of mental disease patients

An unpublished survey of plasma phenylalanine level in approximately 100 patients in mental hospitals and an equal number of controls reveals a slightly higher mean level in the institutionalized population [166] These data (Table 10 12) indicate clearly that most mental hospital

TABLE 10-12 MEAN PLASMA PHENYLALANINE LEVEL IN CONTROL AND MENTAL HOSPITAL POPULATION

| *Subjects* | *No of subjects* | *Mean plasma phenylalanine µmoles/ l ± s d* | *P (vs control)* |
|---|---|---|---|
| Blood donors (controls) | 106 | 0 0[illegible]8 ± 0 015 | |
| Male | 80 | 0 078 ± 0 015 | |
| Female | 6 | 0 080 ± 0 015 | |
| Heterozygotes | 25 | 0 104 ± 0 016 | *<0 001* |
| Male | 12 | 0 108 ± 0 015 | |
| Female | 13 | 0 100 ± 0 016 | |
| Mental patients | 103 | 0 083 ± 0 018 | <0 05 |
| Alcoholics | 24 | 0 088 ± 0 01 | <0 01 |
| Nonalcoholics | 9 | 0 08 ± 0 01[illegible] | <0 1 |
| Male | 43 | 0 08[illegible] ± 0 016 | *<0 001* |
| Female | 3[illegible] | 0 0[illegible] ± 0 01[illegible] | 0 4 to 0 5 |

patients are not phenylketonuric heterozygotes An excess of heterozygotes or some other factor that also gives elevated serum phenylalanine concentrations could account for the higher mean values in the mental hospital population Elevated phenylalanine values were found 15 per cent more commonly in the mental hospital populations than in the control population This is near the maximum estimate from the retrospective studies Some of these higher concentrations occurred in alcoholics in whom liver dysfunction might provide an independent explanation for their high levels The elevated values were not more common in patients over 45 years nor in a specific diagnostic group but they were significantly more common in male patients than in females Such a sex difference did not appear in the controls This recalls Thompson s report of the higher incidence of mental disorder in male relatives of phenylketonuric patients It can only be concluded that hospitalizable mental disorders might occur in as many as one fifth of heterozygotes of phenylketonuria and more frequently in males than females A more precise method must be used to prove that mental disorder is more frequent in heterozygotes of phenylketonuria

## TREATMENT

The therapy of phenylketonuria is considered here only for the confirmation it provides that phenylalanine accumulation is the primary cause of disability in this disease The earlier and useless treatments employing various types of diets and vitamin mineral and hormone supplements will not be considered Attention need be given only to the low phenylalanine regimen The principle of this treatment is to provide only the amount of phenylalanine needed for growth and repair

Phenylalanine limitation was fir t attempted by Dent (unpublished) and by Armstrong and Tyler [8] with diets in which protein was replaced by a costly mixture of pure amino acids L I Woolf (unpublished) suggested a practical method of preparation of casein hydrolyzate with phenylalanine removed by charcoal treatment Bickel et al [7] in 1951 maintained a 2 year-old girl for 6 weeks on a diet based on this preparation Reversal of the major biochemical abnormalities was demonstrated at that time but a larger number of cases and longer periods of treatment have been necessary to evaluate the effect of this diet on the clinical symptomatology These experiments have been possible only because essentially normal growth and development of infants and children can be maintained on this regimen while at the same time the level of phenylalanine in the body is held at the necessarily normal concentration Limitation of phenylalanine intake with more normal diets low in protein have not been successful in either lowering the phenylalanine concentrations or maintaining adequate nutrition [62 106]

The most common difficulty encountered in limiting phenylalanine intake is dietary insufficiency with loss of body protein cessation of growth and flooding of the tissues with phenylalanine released from the body protein In a few cases severe epileptiform seizures were precipitated shortly after the diet was instituted Adequate control depends upon meeting but not exceeding the phenylalanine requirement and meeting the caloric and other nutritional requirements [167] Clinical experience suggests that the phenylalanine requirement estimated at 15 mg per kg per day [167] is too low for young children It may often be two or three times this figure [168 169] Lang Knopp and Weber [170] recommend the higher intake until age 3 The requirement is met by supplying carefully measured amounts of protein such as milk Adequate calories must be supplied from essentially nonprotein sources of carbohydrate and fat along with accessory nutrients The high caloric requirement (more than 100 Cal per kg per day) is apparently associated with the less efficient utilization of free amino acids than of whole proteins Patients appear to do better if a large part of the calories comes from carbohydrates [55]

The degree of biochemical control must be known to evaluate the results of therapy Many patients alternate between the extremes of inadequate restriction and too stringent phenylalanine restriction with weight loss or growth failure Increased phenylalanine plasma concentration and phenylpyruvic acid excretion occur in both situations Since phenylpyruvic acid excretion occurs only at moderately elevated plasma phenylalanine levels continued growth without phenylpyruvic acid excretion can be taken to mark the upper limit of acceptable control Phenylalanine serum elevations short of phenylpyruvic acid excretion may adversely affect the patient The best regulation is possible only with repeated plasma phenylalanine measurements or possibly measurements of urinary o-hydroxyphenylacetic acid or phenylalanine as discussed above under Biochemical Diagnostic Criteria and Methods

*Reversal of Biochemical Abnormalities*

All the major biochemical abnormalities of phenylketonuria are reversed by the low phenylalanine diet Figure 10-13 shows the dramatic effects observed in the first treated case Also illustrated are preliminary periods of treatment showing the effects of several other dietary regimens Besides the decrease of phenylalanine and phenylpyruvic acid *o* hydroxyphenylacetic acid [59] phenylacetylglutamine phenyllactic acid indolyllactic acid and indolylacetic acid [51] decreased to normal values The peculiar odor of the urine vanished There are no reports of the effect of treatment on the supposed excretion of *p*-hydroxyphenyl derivatives or on the sensitivity of these patients to epinephrine The amount of 5-hydroxyindoleacetic acid in urine was unchanged but the serotonin in blood rose during treatment [171]

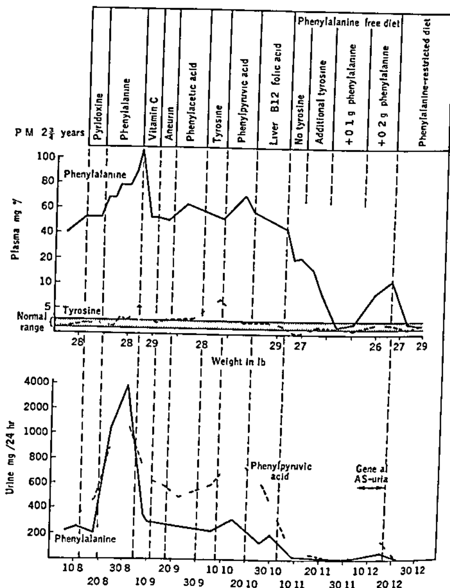



Fig 10-13 The biochemical response of a 3 year-old phenylketonuric girl to various substances and dietary regimens including a phenylalanine free diet The latter reversed the biochemical abnormalities but later caused weight loss and general aminoaciduria (AS-uria) until enough phenylalanine for growth was restored (*By permission of H Bickel et al [53]*)

The abnormal components, which occurred in the β globulin fraction of plasma in about half the cases disappeared with treatment and reappeared when phenylalanine was restored to the diet [87]

*Skin and Pigment Changes*

The eczema of those patients with this disturbance usually cleared promptly during treatment [59 67 168] only to recur if treatment was stopped Hair (and possibly skin) pigmentation has increased with treatment in many individuals This is best seen in the zone of new growth at the hair roots The darkening can also be produced without lowering phenylalanine simply by increasing the tyrosine uptake to overcome the competitive inhibition of tyrosinase by phenylalanine

*Neurologic Signs*

Treatment ameliorates most of the neurologic signs of phenylketonuria with the exception of structural defects and those produced by severe brain damage [59] A prompt effect on the behavior is usually observed The patient becomes less restless and irritable and attention span increases [31] An unmanageable child for example may develop appropriate fear of approaching automobiles Motor performance improves and hyperactive reflexes hyperkinesis and tremors lessen The muscular hypertonicity also decreases When phenylalanine is administered the abnormalities promptly return and large doses intensify this effect

*Seizures and Electroencephalograms*

Severe and almost continuous seizures have been precipitated in the first few weeks of treatment in four cases [8 53 169 170] Two additional cases with seizures and coma one ending fatally were associated with severe hypoglycemia This developed after short fasting on the background of chronic undernutrition associated with low-caloric therapeutic diets [69] The possibility of phenylalanine deficiency as the cause has been suggested

The treatment almost always improves or normalizes the EEG and lessens or eliminates the seizures when continued for a sufficient period The exceptions to this rule are those patients with the most severe neurologic impairment Even in these patients conventional drug therapy may become more effective Except for those with seizures as a complication developing early in treatment no seizures have developed for the first time during therapy Treatment may affect favorably other neurologic signs within weeks but the full effect on the EEG and on seizure frequency is achieved only after months

*Intelligence*

The most important evidence that phenylalanine accumulation is the basic physiologic abnormality in phenylketonuria is the effect of phenyl

TABLE 10-13 PHENYLKETONURIC PATIENTS TREATED WITH LOW PHENYLALANINE DIET WHEN UNDER 2 YEARS OF AGE

| C e | S | Ag t ted | Ag last b erved mo | Chem l c l l | EEG a d geizu es | Devel pm nt | R fer enc |
|---|---|---|---|---|---|---|---|
| 3 | F | 10 d ys– | 12 | Good | | 100 | [172] |
| CM | F | 2 wk– | 5 | Good | | N m l | [168] |
| SR | M | 3 wk– | 5 | Good | | N m l | [59] |
| 1 | F | 4 wk– | 34 | Good | | G Q 88 | [51] |
| — | F | 4 wk | 12 | ? | ? | 94 | [30] |
| 2 | F | 6 wk– | 28 | Good | | | [172] |
| GM | M | 8 wk– | 20 | F | | I Q 90 | [167] |
| 1 | M | 8 wk– | 42 | Good | | D Q 100 | [172] |
| 8 | F | 16 wk– | 16 | G d | | G Q 64 | [41] |
| —* | M | 17 wk– | 30 | Good | ? | I Q 100 | [175] |
| MY | M | 19 wk– | 11 | Good | | N m l | [69] |
| 1808 | F | 6 mo– | 18 | Fa | | D Q 85 | [31] |
| 190P | M | 7 mo– | 31 | Good | | D Q 60 | [31] |
| R D | M | 7½ 29 mo | 42 | Fa | Bec m mal | I Q 83 | [58] |
| 1 | M | 9 mo– | 15 | F | | D Q 22 | [174] |
| MJ | F | 10–15 ( d 17 0 m ) | 37 | Good | B n d mag bn m l EEG fit dec eas | D Q 22 | [59] |
| — | F | 12 mo– | 18 | Fa | EEG abno m l fits? | G Q 19 | [71] |
| J D | F | 13 23 m | 5⁄₁₂ (y ) | Fa | EEG abno m l | D Q 97 H gh gr d o Rx | [69] |
| C M | F | 15 mo– | 24 | Good | Bec me n m l | D Q 60–75 | [58] |
| 11 | M | 15 mo– | 29 | Fa | EEG sl ght imp em t | G Q 32 | [51] |
| 2 | F | 15 mo– | 23 | P | | Id t | [176] |
| 6* | M | 16 mo– | 38 | G d | | G Q 68 | [51] |
| 6 | M | 16–19 mo | 22 | F poor | | M Q 59 | [55] |
| 13UP* | M | 18 mo– | 3 | F | Imp o d EEG | I Q 28 | [31] |
| — | M | 18 mo– | 24 | F poo | F ts l ss f q t | M Q 50 | [67] |
| 5 | F | 20 mo– | 31 | ? | F ts ea d EEG u b g d | D Q 40 | [171] |
| 4 | F | 20 mo– | 34 | ? | | D Q 73 | [171] |
| 9 | F | 20 mo– | 45 | F | EEG bn m l | G Q 31 | [51] |
| 10 | M | 20 mo– | 6 ⁄₁₂ (y ) | Poo | F t c as d | G Q 34 | [51] |
| D W | F | 22 35½ m | 40 | Good | Bec me n m l | (D Q ) 45 | [59] |

N Comm nts EEG d se e g fy wh n b m l t es we e prese t bef tre t- m t Th n q t e t us d t m e d l pm t a r ghly mp ble e f r th y d te test g /ch l gro g × 100 Th d gree f ch m c l o trol is a est m te f m data gi d g d d th d l bas s f th m t f pl asm ph yl l le ls of <0 5 (Good) <1 0 (F ) a d >1 0 (Poo ) μm les per ml du g most f th period. Ph ylpyru acid w ld be ted n th P t lled gr p Case m k d w th a sterisk ( ) we t eated w th Good to Fa c t l d te ted bey d 2 y f g E l f these s tee cases h d l pm tal q ot ts f 80 h gh

alanine deprivation on the intelligence of these patients Reversal of intellectual impairment of older patients has not been realized but the low phenylalanine diet can prevent intellectual impairment during the first few years of life and may reverse recently established impairment [31 51, 55 59 171]

The effects of treatment are in complete accord with the view that the

phenylketonuric infant is normal at birth and that at about 6 months of age retardation begins and becomes progressively more severe The results also indicate that this retardation is largely irreversible although apparently significant improvement has occurred in an occasional child over 3 years of age There were no significant effects in a controlled study of the treatment of adults except that nonintellectual signs were ameliorated [31]

The dramatic effects of treatment are seen in patients diagnosed soon after birth and started on therapy during the first few months of life Most of these have developed normally although the period of observation is still short Gains appear to be maintained fairly well on cessation of therapy Substantial improvement is also seen in children whose treatment is begun as late as 1 or 2 years of age

All reported patients through 1958 whose treatment was started under the age of 2 years appear in Table 10-13 Inadequate biochemical control for shorter or longer periods occurred in many of these patients and some degree of damage was present in the older patients before treatment was started These factors alone could explain the persistence of low developmental quotients in about half Counting only those whose treatment was continued past 2 years of age and only the test results after this age which are less uncertain than earlier tests about half the patients have developmental quotients of above 60 This is in striking contrast to the incidence of only 2 per cent of such high grade patients among untreated phenylketonuric patients The elevation of over half of the early treated patients to the high grade group represents the most conservative estimate of the value and efficacy of the low phenylalanine dietary treatment of phenylketonuria

## SUMMARY

1 The major biochemical changes in phenylketonuria can be traced through known chemical reactions to the accumulation in the tissues of that part of the dietary L-phenylalanine which would normally be converted to tyrosine These changes are reversed when phenylalanine accumulation is avoided The accumulation occurs because the specific liver enzyme phenylalanine hydroxylase is inactive

2 The disease is inherited as an autosomal recessive character The phenylalanine concentration of the serum of heterozygotes may be elevated but there are no definite deleterious phenotypic consequences of the heterozygous state

3 The pathologic changes of skin and mind in phenylketonuria are also referable to the accumulation of phenylalanine since they are either reversed or prevented when the accumulation is avoided by a low phenylalanine diet It is evident that treatment must be started early and

TABLE 10-13 I HENYLKETONURIC PATIENTS TREATED WITH LOW PHENYLALANINE DIET WHEN UNDER 2 YEARS OF AGE

| Ca | S x | Age treated | Age l st bserved mo | Ch mical nt ol | EEG nd eizu | Devel pment | R fer ence |
|---|---|---|---|---|---|---|---|
| 3 | F | 10 d ys– | 12 | Good | | 100 | [17*] |
| CM | F | 2 wk– | 5 | G od | | No m l | [168] |
| SR | M | 3 wk– | 5 | Good | | N m l | [59] |
| 1 | F | 4 wk– | 34 | G d | | G Q 88 | [51] |
| — | F | 4 wk | 12 | ? | ? | 94 | [50a] |
| 2 | F | 6 wk– | 28 | Good | | | [17f] |
| GM | M | 8 wk– | 0 | Fai | | I Q 90 | [16*] |
| 1 | M | 8 wk– | 42 | Good | | D Q 100 | [172] |
| 8 | F | 16 wk– | 16 | Good | | G Q 64 | [51] |
| — | M | 17 wk– | 30 | Go d | ? | I Q 100 | [175] |
| MY | M | 19 wk– | 11 | Good | | N m l | [59] |
| 1808 | F | 6 mo– | 18 | F r | | D Q 85 | [31] |
| 190P | M | 7 n o– | 31 | Good | | D Q 60 | [31] |
| R D | M | 7½ 29 m | 4 | Fa | Bec me n mal | I Q 63 | [59] |
| I | M | 9 mo– | 15 | F | | D Q 22 | [174] |
| MJ | F | 10–15 ( d 17 20 m ) | 37 | G d | B damage b o mal EEG fits d e | D Q 22 | [59] |
| — | F | 12 mo– | 18 | F i | EEG abn rm l fits? | G Q 19 | [71] |
| J Du | F | 13 23 mo | 5½ (yr) | Fa | EEG ab rm l | D Q 97 High-gr d " o R | [59] |
| C M | F | 15 mo– | 24 | Go d | B am n rm l | D Q 60–75 | [59] |
| 11 | M | 15 mo– | 29 | Fa r | EEG light imp ov me t | G Q 32 | [51] |
| | F | 15 mo– | 23 | Poo | | Id t | [175] |
| 6 | M | 16 mo– | 38 | Good | | G Q 68 | [51] |
| 6 | M | 16–19 m | 22 | F r p r | | M Q 59 | [55] |
| 13UP | M | 18 mo– | 32 | F r | Imp d EEG | I Q 28 | [31] |
| — | M | 18 mo– | 24 | F r poor | F ts less freq t | M Q 50 | [67] |
| 5 | F | 0 mo– | 31 | ? | F t ased EEG h ng d | D Q 40 | [171] |
| 4* | F | 0 mo– | 34 | ? | | D Q 73 | [171] |
| 9 | F | 0 mo– | 45 | F | EEG ab m l | G Q 31 | [51] |
| 10 | M | 0 mo– | 6½ (yr) | Poor | F ts as d | G Q 34 | [51] |
| D W | F | 2 35½ m | 40 | Good | Bec m norm l | (D Q ) c 45 | [59] |

NOTE Comm nts on EEG nd se u es a g o ly when b malit w p es nt bef tre ment The v rιou q t t d t m s d l pm t a ughly c mp bl n s f as th y nd c te te t ge/ch l g g × 100 The d gr e f h m al c trol eat m te Ir m data g e and grad d on the deal bas f th t c f pla m ph ylal m e l la f <0 5 (Good) <1 0 (Fa r) a d >1 0 (P r) μm l p ml d g m t f th p d Phe ylpyru c ac d would be ex t d ın the Poo t lled g p Cas m k d with n asterisk ( ) we t eated w th Good to Fair at l d test d bey d y of ge El n f these ει tee ases b d l pm tal q oti ts of 60 h gh

alanine deprivation on the intelligence of these patients Reversal of intellectual impairment of older patients has not been realized but the low phenylalanine diet can prevent intellectual impairment during the first few years of life and may reverse recently established impairment [31 51 55, 59 171]

The effects of treatment are in complete accord with the view that the

ketonuric defect since phenylalanine hydroxylase appears for the first time during the biochemical differentiation of the liver following birth It is the failure to develop phenylalanine hydroxylase activity that is hereditary

5 The consequences of the defect are best considered as the results of accumulation of (internal) environmental abnormalities which affect the tissues of the developing child Some of these may be enzyme inhibitors and pharmacologic effects on preformed systems Others may involve adaptive changes in the capacity of systems to handle unusual amounts of metabolites All these should be and are found to be reversible but those systems which still have to undergo biochemical differentiation when the chemical milieu becomes abnormal may be directed along an irreversible series of abnormal changes

6 Too little is now known of the nature and control of embryologic and biochemical differentiation for understanding the intimate cellular consequences of the phenylketonuric abnormality but when the chemical milieu becomes abnormal the brain is one of the few organs which must still differentiate biochemically and it is irreversibly altered by this disease The retardation of myelination is the only evidence besides functional tests that the brain is affected

7 It is possible that late dentition and enamel hypoplasia also reflect the effects of the abnormal chemical milieu Possibly other differentiations are altered but the evidence is not convincing enough to include such items as hypogenitalism which are sometimes mentioned Unspecified developmental abnormalities of a functional sort may contribute to the decreased survival of these patients The outstanding problem in phenylketonuria is no longer the effect of the abnormal metabolism on the brain but the nature of the alterations induced in the developing brain

## BIBLIOGRAPHY


1 Følling A Über Ausscheidung von Phenylbrenztraubensaure in den Harn als Stoffwechselanomalie in Vergindung mit Imbezillität Ztschr physiol Chem **227** 169 1934

2 Garrod A E *Inborn Errors of Metabolism* Oxford London 1909

3 Jervis G A The genetics of phenylpyruvic oligophrenia J Ment Sc **85** 719 1939

4 Jervis G A Block R J Bolling D and Kanze L Phenylalanine content of blood and spinal fluid in phenylpyruvic oligophrenia J Biol Chem **134** 105 1940

5 Jervis G A Studies of phenylpyruvic oligophrenia the position of the metabolic error J Biol Chem **169** 651 194

6 Jervis G A Phenylpyruvic oligophrenia deficiency of phenylalanine oxidizing system Proc Soc Exper Biol & Med **82** 514 1953

7 Bickel H Gerrard J and Hickmans E M Influence of phenylalanine intake on the chemistry and behavior of a phenylketonuric child Acta pediat **43** 64 1954

8 Armstrong M D and Tyler F H Studies on phenylketonuria I Restricted phenylalanine intake in phenylketonuria J Clin Invest **34** 565 1955

continued at least until biochemical differentiation is complete The step wise pathogenesis of the serious and permanent mental deficiency and other defects is less well understood than the biochemical disturbances One of the abnormalities, melanin deficiency, is clearly referable

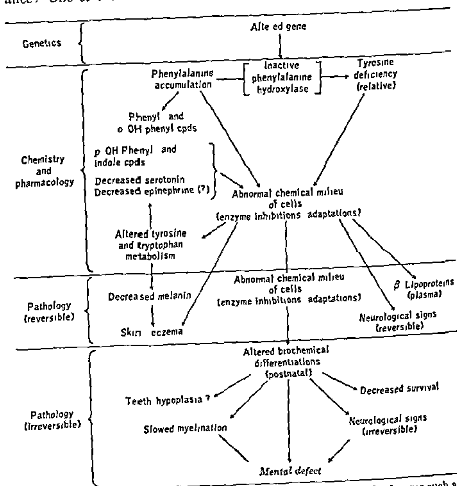



Fig 10-14 Scheme of pathogene is in phenylketonuria Irreversible changes such a the mental defect are considered to result from altered biochemical differentiation of the immature brain

to competitive enzyme inhibition Pharmacologic effects of the abnormal metabolites especially derivatives of *o*-tyrosine and serotonin may contribute to the symptomatology The pathogenesis involving the chemical derangements and the reversible and irreversible pathologic changes is summarized in Fig 10 14

4 The disease phenylketonuria but not the primary defect is acquired Before and immediately after birth all animals have the ' phenyl

33 Scholz W Contribution à l anatomie pathologique du système nerveux central dans l oligophrénie phenylpyruvique Encéphale 46 668, 1957
34 Penrose L S Peripheral nerve tumors in a case of phenylketonuria Lancet 1 572 1939
35 Coquet M Myle G Nyssen R and Van Bogaert L. Première observation de l oligophrénie phenylpyruvique Monatsschr Psychiat u Neurol 109 133 1944
36 Fellman J H Epinephrine metabolites and pigmentation in the central nervous system in a case of phenylpyruvic oligophrenia J Neurol Neurosurg Psychiat 21 58 1958
37 Leoni G Contributo alla conoscenza dell anatomia pathologica del morbo di Følling od oligofrenia fenil piruvica Gior psichiat e neuropat 81 775 1953
38 Cowie V Phenylpyruvic oligophrenia J Ment Sc 97 505 1951
39 Larson C A Phenylpyruvic oligophrenia in Swedish institutionalized mental defectives Hereditas 36 109 1950
40 Benda C E In *Developmental Disorders of Mentation and Cereb al Palsies* p 459 Grune & Stratton New York 1952
41 Mautner H and Quinn K V Phenylpyruvic oligophrenia Ann paediat (Basel) 172 1 1940
42 Corsellis J A N The pathological report of a case of phenylpyruvic oligophrenia J Neurol Neurosurg & Psychiat 16 139 1953
43 Josephy H Phenylpyruvica ol gophrenia Illinois M J 94 107 1948
44 Sander G *Zur pathologischen Anatomie und Pathophysiologie des Gehirns bei der Phenylpyruvisch en Idiotie* Inaugural dissertation Zurich 1951
45 Klein D Über einen Fall von phenylpyruvischen Idiotie mit Zwergwuchs (Zurgleich ein Beitrag zum Problem der motorischen Phänomene bei deser Schwachsinnsform) Monatsschr Psychiat u Neurol 111 273 1946
46 Meister P Die Oligophrenia phenylpyruvica eine sonder Form der chronischen Aminoacidurie Helv pa diatr acta 6 504 1951
47 Paine R S Variability in manifestations of untreated patients with phenyl ketonuria Pediatrics 20 290 1957
48 Cowie V and Penrose L S Dilution of hair colour in phenylketonuria Ann Eugenics 15 297 1951
49 Coates S Norman A P and Woolf L I Phenylketonuria with normal intelligence and Gowen s muscular dystrophy Arch Dis Childhood 32 313 1957
50 Low N L, Armstrong M D and Carlisle J W Phenylketonuria two unusual cases Lancet 2 917 1956 (cf Table 10-4)
51 Woolf L I Griffiths R Moncrieff A Coates S and Dillistone F Dietary treatment of phenylketonuria Arch Dis Childhood 33 167 1958
52 Bickel H and Grüter W Phenylketonuria with normal I Q Comparison of biochemical and psychodiagnostic tests of two girls Ztschr Kinderh 79 509 1957
53 Bickel H Boscott R J, and Gerrard J In *Biochemistry of the Develop g Nervo s Sy tem* p 475 Academic Pres Inc New York 1955
54 Lang K Über phenylpyruvische Oligophrenia Ztschr Kinderh 75 132 1954
55 Blainey J D and Gulliford R. Phenylalanine restricted diets in the tre tment of phenylketonuria Arch Dis Childhood 31 452 1956
56 Leonard S A and McGuire F L Phenylketonuria an unusual case J Pediat 54 210 1959
57 Brugg r C Die Ausscheidung von Phenylbrenztraubensaure in Urin von Schwa h inn gen Schw rz Arch N urol u Psychiat 49 67 1942
58 Hsia D Y Knox W E and Paine R. S A case of phenylketonuria with borderl ne intell gence A M A Am J Dis Chil 94 33 1957
59 Armstrong M D Low N L and Bosma, J F Stud es on phenylketonuria IX Further observations on the effect of phenylalanine-restricted diet on patients with phenylketonuria Am J Clin Nutrition 5 543 195
60 Knox W E and Messinger E. The d tect on of the metabol effect of the recessive gene for phenylketonuria. Am J Human Genet 10 53 1958

9 Hsia D Y Driscoll K Troll W and Knox W E Detection by phenylalanine tolerance tests of heterozygous carriers of phenylketonuria Nature London 178 1239 1956
10 Følling A Mohr O L and Ruud L *Oligophrenia phenylpyruvica a Recessive Syndrome in Man Skifter Utgitt av Det Norske Videnskaps Akademi I Oslo I* Mat Naturv Klasse 1944 Bind 2 No 13 44 pp Dybwad Oslo 1945
11 Vizet J *L acide phenylpyruvique* Thesis Faculté de médicine et pharmacie Université de Bordeaux 1953
12 Jervis G A Phenylpyruvic oligophrenia Res Publ A Nerv & Ment Dis 33 259 1954
13 Lang K Die phenylpyruvische Oligophrenia Ergebn inn Med u Kinderh 6 78 1955
14 Knox W E and Hsia D Y Pathogenetic problems in phenylketonuria Am J Med 22 687 1957
15 Wright S W and Tarjan G Phenylketonuria A M A Am J Dis Child 93 405 1957
16 Penrose L and Quastel J H Metabolic studies in phenylketonuria Biochem J 31, 266 1937
17 Munro T A Phenylketonuria data of 47 British families Ann Eugenics 14 60 1947
18 Larson C A An estimate of the frequency of phenylketonuria in South Sweden Folia héréd et path 4 40 1954
19 Larson C A The frequency of first cousin marriages in a south Swedish rural community Am J Human Genet 8 151 1956
20 Armstrong M D and Low N L Phenylketonuria VIII Relation between age serum phenylalanine level and phenylpyruvic acid excretion Proc Soc Exper Biol & Med 94 142 1957
21 Jervis G A Phenylpyruvic oligophrenia Arch Neurol & Psychiat 38 944 1937
22 Turpin R Dagand H Duchene H and Delbarre F Présentation clinique d un malade atteint d oligophrénie phenylpyruvique Ann Med Psychol 105 65 1947
23 Cohen P and Kozinn P J Phenylpyruvic oligophrenia in a Jewish child J Pediat 34 76 1949
24 Ferriera Fernándes J Oligofrenia fenilpirúvica en melanoderma Brasil méd 64 2?5 1950
25 Stadler H E Meyer H and Leland H Phenylpyruvic oligophrenia in a mulatto J Nerv & Ment Dis 124 205 1956
26 Utena H and Saito T On the phenylpyruvic oligophrenia Psychiatria et Neurologia Japonica 53 365 1951
27 Kobayashi T Saito S and Kamamura K Oligophrenia pyruvica three cases in one family Shônika Rinshô 5 6 1952
28 Bhaskaran K Phenylpyruvic oligophrenia A short review with the report of an affected family Indian M Gaz 87 506 1952
29 Larson C A The absence of phenylketonuria in 8220 individuals not known to be mental defectives in *Novant anni delle Leggi Mendeliane* p 311 Instituto Gregorio Mendel Rome 1956
30 Schonenberg H Schmidt D and Wonckhaus I Der phenylbrenz Traubensaureschwachsinn Ztschr Kinderh 77 363 1955
30a Gibbs N K and Woolf L I Tests for phenylketonuria Brit M J Sept 26 1959
31 Hsia D Y Knox W E Quinn K V and Paine R S A one year controlled study of the effect of low phenylalanine diet on phenylketonuria Pediatrics 21 178 1958
32 Alvord E C Stevenson L D Vogel F S and Engle R L Jr Neuropathological findings in phenyl pyruvic oligophrenia J Neuropath & Exper Neutrol 9 298 1950
32a Hassel C W and Brunsting L A Phenylpyruvic oligophrenia A M A Arch Dermat & Syph 79 458 1959

86 Borek F Brecher A Jervis C A and Waelsch H Oligophrenia phenylpyruvica constancy of the metabolic error Proc Soc Exper Biol & Med 75 86 1950
87 Brown D M Armstrong M D and Smith E D Studies on phenylketonuria IV Serum proteins in phenylketonuria Proc Soc Exper Biol & Med 89 367 1955
88 Jervis G A Excretion of phenylalanine and derivatives in phenylpyruvic oligophrenia Proc Soc Exper Biol & Med 75 83 1950
89 Jervis G A Studies on phenylpyruvic oligophrenia phenylpyruvic acid content of blood Proc Soc Exper Biol & Med 81 715 1952
90 Pare C M B Sandler M and Stacey R S 5-Hydroxytryptamine deficiency in phenylketonuria Lancet 1 511 1957
91 Armstrong M D Shaw K N F and Robinson K S Studies on phenylketonuria II The excretion of o-hydroxyphenylacetic acid in phenylketonuria J Biol Chem 213 797 1955
92 Armstrong M D and Robinson K S On the excretion of indole derivatives in phenylketonuria Arch Biochem & Biophys 52 287 1954
93 Woolf L I Excretion of conjugated phenylacetic acid in phenylketonuria Biochem J 49 ix 1951
94 Henry R J Sobel C and Chiamori N Method for determination of serum phenylalanine with use of the Kapellar Adler reaction A M A Am J Dis Child 94 604 195
95 Zahn H and Marstaller H Phenylalanine in serum Ztschr physiol Chem 310 44 1958
96 Weil Malherbe H The level of adrenalin in human plasma and its relation to mental activity J Ment Sc 101 733 1955
97 Mitoma C Posner H S Bogdanski D F and Udenfriend S Biochemical and pharmacological studies on o-tyrosine and its meta and para analogues a suggestion concerning phenylketonuria J Pharmacol & Exper Therap 120 188 1957
98 Zeller E A Isolierung von Phenylmilchsaure und Phenyltraubensaure aus Harn bei Imbecillitas phenylpyruvica Helvet chim acta 26 1614 1943
99 Fowler D I Norton P M Cheung M W and Pratt F L Observations on urinary amino acid excretion in man the influence of age and diet Arch Biochem & Biophys 68 452 1957
100 Jepson J B Paper chromatography of urinary indoles Lancet 2 1009 1955
101 Ferrari V Campagnari F and Guida A Oligofrenia fenilpiruvica nuovi reperti clinico-pathologici Minerva Med 46 119 1955
102 Stein W H Paladini A C Hirs C H and Moore S Phenylacetylglutamine as a constituent of normal human urine J Am Chem Soc 76 2848 1954
103 Meister A Udenfriend S and Bessman S P Diminished phenylketonuria in phenylpyruvic oligophrenia after administration of L-glutamine L-glutamate or L-asparagine J Clin Invest 35 619 1956
104 Russo H F Wright L D Skeggs H R Tillson E K and Beyer K H Renal clearances of essential amino acids threonine and phenylalanine Proc Soc Exper Biol & Med 66 215 1947
105 Doolan P D Harper H A Hutchin M F and Shreeve W W Renal clearance of 18 individual amino acids in human subjects J Clin Invest 34 1247 1955
106 Berry H K Sutherland B S Guest G M and Umbarger B Chemical and clinical observations during treatment of children with phenylketonuria Pediatrics 21 929 1958
107 Cullen A M and Knox W E o-Hydroxyphenylacetic acid excretion in the phenylalanine tolerance test for carriers of phenylketonuria Proc Soc Exper Biol & Med 99 219 1958
108 Sherwin C P and Kennard K S Toxicity of phenylacetic acid J Biol Chem 40 259 1919
109 Sherwin C P Wolf M and Wolf W The maximum production of glutamine

61 Penrose L S A search for linkage between the A B O agglutinogens and phenylketonuria Am J Ment Deficiency 50 4 1945
62 Meyer N Mertz L T Stadler H J Leland H and Colandro J Psychometabolic changes in phenylketonuria treated with low phenylalanine diet A M A Arch Int Med 101 1094 1958
63 Harris H An Introduction to Human Biochemical Genetics Eugenics Laboratory Memoirs XXXVII Cambridge University Press London 1955
64 Woolf L I and Vulliamy D G Phenylketonuria with a study of the effect upon it of glutamic acid Arch Dis Childhood 26 487 1951
65 Berg J M and Stern J Iris color in phenylketonuria Ann Human Genet 22 370 1958
66 Fois A Rosenberg C and Gibbs F A The electroencephalogram in phenylpyruvic oligophrenia Electroencephalog Clin Neurophysiol 7 569, 1955
67 Braude H Phenylketonuria—a case report in a European child treated with low phenylalanine diet South African M J 30 83 1956
68 Peters G Die Storung des Lipoid Kohlenhydrat und Eiweisstoffwechsels in Handbuch d Sperz Path Anat u Histol edited by O Lubarsch F Henke and R Rossle Band XIII Part 2 p 1889 Springer Berlin 1958
69 Dodge P R Mancall E L Crawford J D Knapp J and Paine R S Hypoglycemia complicating treatment of phenylketonuria with a phenylalanine-deficient diet report of two cases New England J Med 260 1104 1959
70 Folch Pi J Composition of the brain in relation to maturation in Biochemistry of the Developing Nervous System edited by H Waelsch p 121 Academic Press Inc New York 1955
71 Fullon D H H Craniolacunia and phenylpyruvic oligophrenia Brit J Radiol 31 634 1958
72 Smith A J and Strang L B Inborn errors of metabolism with urinary excretion of α-hydroxy butyric acid and phenylpyruvic acid Arch Dis Childhood 33 109 1958
73 Kotake Y Masai Y and Mori Y Über das Verhalten des Phenylalanins im tierischen Organismus Ztschr physiol Chem 122 195 1922
74 Chandler J P and Lewis H B Comparative studies of the metabolism of the amino acids J Biol Chem 96 619 1932
75 Prescott A B Borek E Brecher A and Waelsch H Studies on oligophrenia phenylpyruvica I J Biol Chem 181 273 1949
76 Schreier K and Flaig H Urinary excretion of indole pyruvic acid in normal conditions and in Følling s disease Klin Wchnschr 34 1213 1956
77 Fellman J H Detection of phenylpyruvic oligophrenia J Nerv & Ment Dis 123 575 1956
78 Berry J P and Woolf L I Estimation of phenylpyruvic acid Nature London 169 202 1952
78a Saifer A and Harris A Studies on the photometric determination of phenylpyruvic acid in urine Clin Chem 5 203 1959
79 Følling A Closs K and Gamnes T Vorlaufige Schlus folg rung n aus Belastungsversuchen mit Phenylalanin an Menschen und Tieren Ztschr physiol Chem 256 1 1938
80 Stein W H Bearn A G and Moore S The amino acid content of the blood and urine in Wilson s disease J Clin Invest 33 410 1954
81 Evered D F The excretion of amino acids by the human Biochem J 62 416 1956
82 Stein W H and Moore S The free amino acids of human blood plasma J Biol Chem 211 915 1954
83 Hier S W and Bergeim O The microbiological determination of certain free amino acids in human and dog plasma J Biol Chem 163 129 1946
84 Harper H A Hutchin M E and Kimmel J R Concentrations of 19 amino acids in plasma and urine of fasting normal males Proc Soc Exper Biol & Med 80 768 1952
85 Berry H Sutherland B S and Guest G M Phenylalanine tolerance tests on relatives of phenylketonuric children Am J Human Genet 9 310 1957

86 Borek I Brecher A Jervis C A and Waelsch H Oligophrenia phenylpyruvica constancy of the metabolic error Proc Soc Exper Biol & Med 75 86 1950
87 Brown D M Armstrong M D and Smith L D Studies on phenylketonuria IV Serum proteins in phenylketonuria Proc Soc Exper Biol & Med 89 367 1955
88 Jervis G A Excretion of phenylalanine and derivatives in phenylpyruvic oligophrenia Proc Soc Exper Biol & Med 75 83 1950
89 Jervis G A Studies on phenylpyruvic oligophrenia phenylpyruvic acid content of blood Proc Soc Exper Biol & Med 81 715 1952
90 Pare C M B Sandler M and Stacey R S 5-Hydroxytryptamine deficiency in phenylketonuria Lancet 1 511 1957
91 Armstrong M D Shaw K N F and Robinson K S Studies on phenylketonuria II The excretion of o-hydroxyphenylacetic acid in phenylketonuria J Biol Chem 213 797 1955
92 Armstrong M D and Robinson K S On the excretion of indole derivatives in phenylketonuria Arch Biochem & Biophys 52 287 1954
93 Woolf L I Excretion of conjugated phenylacetic acid in phenylketonuria Biochem J 49 ix 1951
94 Henry R J Sobel C and Chiamori N Method for determination of serum phenylalanine with use of the Kapellar Adler reaction A M A Am J Dis Child 94 604 1957
95 Zahn H and Marstaller H Phenylalanine in serum Ztschr physiol Chem 310 44 1958
96 Weil Malherbe H The level of adrenaline in human plasma and its relation to mental activity J Ment Sc 101 733 1955
97 Mitoma C Posner H S Bogdanski D F and Udenfriend S Biochemical and pharmacological studies on o-tyrosine and its meta and para analogues a suggestion concerning phenylketonuria J Pharmacol & Exper Therap 120 188 1957
98 Zeller E A Isolierung von Phenylmilchsaure und Phenyltraubensaure aus Harn bei Imbecillitas phenylpyruvica Helvet chim acta 26 1614 1943
99 Fowler D I Norton P M Cheung M W and Pratt E L Observations on urinary amino acid excretion in man the influence of age and diet Arch Biochem & Biophys 68 452 1957
100 Jepson J B Paper chromatography of urinary indoles Lancet 2 1009 1955
101 Ferrari V Campagnari F and Guida A Oligofrenia fenilpiruvica nuovi reperti chimico pathologici Minerva Med 46 119 1955
102 Stein W H Paladini A C Hirs C H and Moore S Phenylacetylglutamine as a constituent of normal human urine J Am Chem Soc 76 2848 1954
103 Meister A Udenfriend S and Bessman S P Diminished phenylketonuria in phenylpyruvic oligophrenia after administration of L-glutamine L-glutamate or L-asparagine J Clin Invest 35 619 1956
104 Russo H F Wright L D Skeggs H R Tillson E K and Beyer K H Renal clearances of essential amino acids threonine and phenylalanine Proc Soc Exper Biol & Med 65 215 1947
105 Doolan P D Harper H A Hutchin M E and Shreve W W Renal clearance of 18 individual amino acids in human subjects J Clin Invest 34 1247 1955
106 Berry H K Sutherland B S Guest G M and Umbarger B Chemical and clinical observation during treatment of children with phenylketonuria Pediatrics 21 929 1958
107 Cullen A M and Knox W E o-Hydroxyphenylacetic acid excretion in the phenylalanine tolerance test for carriers of phenylketonuria Proc Soc Exper Biol & Med 99 219 1958
108 Sherwin C P and Kennard K S Toxicity of phenylacetic acid J Biol Chem 40 259 1919
109 Sherwin C P Wolf M and Wolf W The maximum production of glutamine

by the human body as measured by the output of phenylacetylglutamine J Biol Chem 37 113 1919
110 Bruns F H and Fiedler L Enzymatic cleavage and synthesis of L threo-β phenylserine and L-erythro β phenylserine Biochem Ztschr 330 324 1958
111 Armstrong M D Chao F, Parker V J and Wall J T Endogenous formation of hippuric acid Proc Soc Exper Biol & Med 90 675 1955
112 Moss, A R and Schoenheimer R The conversion of phenylalanine to tyrosine in normal rats J Biol Chem 135 415 1940
113 Udenfriend S The hydroxylation of phenylalanine and antipyrine in phenylpyruvic oligophrenia J Biol Chem 203 961 1953
114 Mitoma C Auld R M and Udenfriend S On the nature of enzymic defect in phenylpyruvic oligophrenia Proc Soc Exper Biol & Med 94 634 1957
115 Wallace H W Moldave K and Meister A Studies on conversion of phenylalanine to tyrosine in phenylpyruvic oligophrenia Proc Soc Exper Biol & Med 94 632 1957
116 Kaufman S Phenylalanine hydroxylation cofactor in phenylketonuria Science 128 1506 1958
117 Kenney F T Reem G H and Kretchmer N Development of phenylalanine hydroxylase in mammalian liver Science 127 86 1958
118 Kaufman S The participation of tetrahydrofolic acid in enzymic conversion of phenylalanine to tyrosine Biochim et biophys acta 27 428 1958
119 Knox W E The degradation of aromatic compounds in *Spon's Handbook* London (in press)
120 Udenfriend S Clarke C T Axelrod J and Brodie B B Ascorbic acid in aromatic hydroxylation I A model system for aromatic hydroxylation J Biol Chem 208 731 1954
121 Fellman J H and Devlin M K Concentration and hydroxylation of free phenylalanine in adrenal glands Biochim et biophys acta 28 328 1958
122 Schmidt F and Klinger W Untersuchungen über Hydroxylierungen an Phenylalkylderivaten durch Ascorbinsäure Ztschr physiol Chem 310 3 1958
123 Knox W E The metabolism of phenylalanine and tyrosine in *Symposium on Amino Acid Metabolism* edited by W D McElroy and H B Glass p 836 Johns Hopkins Press Baltimore 1955
124 Lin E C C Pitt B M Civen M and Knox W E The assay of aromatic amino acid transaminations and keto acid oxidation by the enol borate tautomerase method J Biol Chem 233 668 1958
125 Canellakis Z N and Cohen P P Kinetic and substrate specificity study of tyrosine-α ketoglutaric acid transaminase J Biol Chem 222 63 1956
126 Lin E C C and Knox W E Specificity of the adaptive response of tyrosine-α-ketoglutarate transaminase in the rat J Biol Chem 233 1186 1958
127 LaDu B N and Zannoni V G The tyrosine oxidation system of liver III Further studies on the oxidation of p-hydroxyphenylpyruvic acid J Biol Chem 219 273 1956
128 Hager S E Gregerman R I and Knox W E p-Hydroxyphenylpyruvate oxidase of liver J Biol Chem 225 935 1957
129 Udenfriend S and Mitoma C In *Symposium on Amino Acid Metabolism* edited by W D McElroy and H B Glass p 876 Johns Hopkins Press Balti*more* 1955
130 Armstrong M D and Shaw K N F Studies on phenylketonuria III The metabolism of *o*-tyrosine J Biol Chem 213 805 1955
131 Flatow L Über den Abbau von Aminosäuren im Organismus Ztschr physiol Chem 64 367 1910
132 Blaschko H The decarboxylation of *o*-hydroxyphenylalanine Biochem J 44 268 1949
133 Fellman J H Inhibition of DOPA decarboxylase by aromatic amino acids associated with phenylpyruvic oligophrenia Proc Soc Exper Biol & Med 93 413 1956
134 Randall L D *Oxidation of phenethylamine derivatives by amine oxidase* J Pharmacol 88 216 1946

135 Lin E C C Civen M and Knox W E Effect of vitamin $B_6$ deficiency on the basal and adapted levels of rat liver tyrosine and tryptophan transaminase J Biol Chem 233 1183 1958
136 Armstrong M D and Binkley E L Jr Studies on phenylketonuria V Observations on a newborn infant with phenylketonuria Proc Soc Exper Biol & Med 93 418 1956
137 Auerbach V H and Waisman H A Tryptophan peroxidase-oxidase histidase and transaminase activity in the liver of the developing rat J Biol Chem 234 304 1959
138 Meister A Reduction of α- γ-diketo and α keto acids catalyzed by crystalline lactic dehydrogenase J Biol Chem 184 117 1950
139 Moldave K and Meister A Synthesis of acetylglutamine by human tissue J Biol Chem 229 463 1957
140 Meister A Phenylpyruvic oligophrenia Pediatrics 21 1021 1958
141 Mehler A H In *Symposium on Amino Acid Metabolism* edited by W D McElroy and H B Glass p 878 Johns Hopkins Press Baltimore 1955
142 Udenfriend S Weissbach H and Bogdansky D F Increase in tissue serotonin following administration of its precursor 5-hydroxytryptophan J Biol Chem 224 803 1957
143 Knox W E and Mehler A H The conversion of tryptophan to kynurenine in liver I The coupled tryptophan and peroxidase-oxidase system forming formylknurenine J Biol Chem 187 419 1950
144 Pare C M B Sandler M and Stacey R S Decreased 5-hydroxytryptophan decarboxylase activity in phenylketonuria Lancet 2 1099 1958
145 Davison A N and Sandler M Inhibition of 5-hydroxytryptophan decarboxylase by phenylalanine metabolites Nature London 181 186 1958
146 Bickis I J Kennedy J P and Quastel J H Phenylalanine inhibition of tyrosine metabolism in the liver Nature London 179 1124 1957
147 Dancis J and Balis M E A possible mechanism for disturbance in tyrosine metabolism in phenylpyruvic oligophrenia Pediatrics 15 63 1955
148 Miyamoto M and Fitzpatrick T B Competitive inhibition of mammalian tyrosinase by phenylalanine and its relationship to hair pigmentation in phenylketonuria Nature London 179 199 1957
149 Snyderman S E Norton P and Holt L E Jr Effect of tyrosine administration in phenylketonuria Fed Proc 14 450 1955
150 Funkinstein D H Greenblatt M and Solomon H C Autonomic changes in paralleling psychologic changes in mentally ill patients J Nerv & Ment Dis 114 1 1951
151 Cawte J E Phenylketonuria M J Australia 2 15 1954
152 Cawte J E Attempt to determine reaction to adrenaline in phenylketonuria J Ment Deficiency Res 1 111 1957
153 Allen D W and Schroeder W A A comparison of the phenylalanine content of hemoglobin of normal and phenylketonuric individuals determined by ion exchange chromatography J Clin Invest 36 1343 1957
154 Himwich H E and Fazekas J F Cerebral metabolism in mongolian idiocy and phenylpyruvic oligophrenia Arch Neurol & Psychiat 44 1213 1940
155 Himwich H E and Fazekas J F Cerebral arteriovenous oxygen difference II Mental deficiency Arch Neurol & Psychiat 51 73 1944
156 Auerbach V H and Waisman H A The metabolism of aromatic amino acids in leukemic rats Cancer Res 18 536 1958
157 Auerbach V H Waisman H A and Wycoff L B Phenylketonuria in the rat associated with decreased temporal discrimination learning Nature London 182 871 1958
158 Brecher R and Brecher E Saving Children from mental retardation Sat Eve Post 232 3 1959
159 Thompson T H Relatives of phenylketonuric patients J Ment Deficiency Res 1 67 1957
160 Penrose L S The inheritance of phenylpyruvic amentia (phenylketonuria) Lancet 2 192 1935

by the human body as measured by the output of phenylacetylglutamine J Biol Chem 37 113 1919
110 Bruns F H and Fiedler L Enzymatic cleavage and synthesis of L-threo-β phenylserine and L-erythro β phenylserine Biochem Ztschr 330 324 1958
111 Armstrong M D Chao F Parker V J and Wall I E Endogenous formation of hippuric acid Proc Soc Exper Biol & Med 90 675 1955
112 Moss A R and Schoenheimer R The conversion of phenylalanine to tyrosine in normal rats J Biol Chem 135 415 1940
113 Udenfriend S The hydroxylation of phenylalanine and antipyrine in phenyl pyruvic oligophrenia J Biol Chem 203 961 1953
114 Mitoma C Auld R M and Udenfriend S On the nature of enzymic defect in phenylpyruvic oligophrenia Proc Soc Exper Biol & Med 94 634 1957
115 Wallace H W Moldave K and Meister A Studies on conversion of phenyl alanine to tyrosine in phenylpyruvic oligophrenia Proc Soc Exper Biol & Med 94 632 1957
116 Kaufman S Phenylalanine hydroxylation cofactor in phenylketonuria Science 128 1506 1958
117 Kenney F T Reem G H and Kretchmer N Development of phenyl alanine hydroxylase in mammalian liver Science 127 86 1958
118 Kaufman S The participation of tetrahydrofolic acid in enzymic conversion of phenylalanine to tyrosine Biochim et biophys acta 27 428 1958
119 Knox W E The degradation of aromatic compounds in *Spon s Handbook* London (in press)
120 Udenfriend S Clarke C T Axelrod J and Brodie B B Ascorbic acid in aromatic hydroxylation I A model system for aromatic hydroxylation J Biol Chem 208 731 1954
121 Fellman J H and Devlin M K Concentration and hydroxylation of free phenylalanine in adrenal glands Biochim et biophys acta 28 328 1958
122 Schmidt F and Klinger W Untersuchungen uber Hydroxylierungen an Phenylalkylderivaten durch Ascorbinsaure Ztschr physiol Chem 310 3 1958
123 Knox W E The metabolism of phenylalanine and tyrosine in *Symposium on Amino Acid Metabolism* edited by W D McElroy and H B Glass p 836 Johns Hopkins Press Baltimore 1955
124 Lin E C C Pitt B M Civen M and Knox W E The assay of aromatic amino acid transaminations and keto acid oxidation by the enol borate tautomerase method J Biol Chem 233 668 1958
125 Canellakis Z N and Cohen P P Kinetic and substrate specificity study of tyrosine α ketoglutaric acid transaminase J Biol Chem 222 63 1956
126 Lin E C C and Knox W E Specificity of the adaptive response of tyrosine-α-ketoglutarate transaminase in the rat J Biol Chem 233 1186 1958
127 LaDu B N and Zannoni V G The tyrosine oxidation system of liver III Further studies on the oxidation of p-hydroxyphenylpyruvic acid J Biol Chem 219 273 1956
128 Hager S E Gregerman R I and Knox W E p Hydroxyphenylpyruvate oxidase of liver J Biol Chem 225 935 1957
129 Udenfriend S and Mitoma C In *Symposium on Amino Acid Metabolism* edited by W D McElroy and H B Glass p 876 Johns Hopkins Press Baltimore 1955
130 Armstrong M D and Shaw K N F Studies on phenylketonuria III The metabolism of o-tyrosine J Biol Chem 213 805 1955
131 Flatow L Über den Abbau von Aminosauren im Organismus Ztschr physiol Chem 64 367 1910
132 Blaschko H The decarboxylation of o-hydroxyphenylalanine Biochem J 44 268 1949
133 Fellman J H Inhibition of DOPA decarboxylase by aromatic amino acids associated with phenylpyruvic oligophrenia Proc Soc Exper Biol & Med 93 413 1956
134 Randall L D Oxidation of phenethylamine derivatives by amine oxidase J Pharmacol 88 216 1946

# Chapter 11

## Tyrosinosis

*Bert N La Du*

Classification of tyrosinosis as one of the inborn errors of metabolism is rather presumptive in view of the fact that only one case has ever been described and the inheritability of this condition has not yet been demonstrated Nevertheless tyrosinosis has been generally accepted as a *bona fide* addition to the group of metabolic diseases associated with defects at particular steps in phenylalanine and tyrosine metabolism i e albinism phenylketonuria and alcaptonuria [*1* *2*]

In 1927 Medes Berglund and Lohmann [*3*] found an unusual reducing substance in the urine of a patient with myasthenia gravis The reducing compound was later isolated and identified as *p* hydroxyphenylpyruvic acid the α keto acid of tyrosine and this condition was named *tyrosinosis* by Medes [*4*] Other patients with myasthenia gravis were not found to excrete *p* hydroxyphenylpyruvic acid and no particular clinical symptoms were observed in the tyrosinosis patient which could be attributed to the excretion of the α keto acid Without doubt tyrosinosis must be a very rare disease A search for additional cases made by Blatherwick among more than 20 000 persons with a weakly positive reduction test result for glucose in the urine failed to uncover any additional cases [*5*]

### METABOLIC STUDIES IN TYROSINOSIS PATIENT

It is indeed fortunate that the unique case of tyrosinosis was in the hands of the capable investigator Grace Medes her classic paper in 1932 [*4*] still remains the key source of information on this disease The patient with tyrosinosis was a male Russian Jew 49 years old at the time Initial metabolic balance studies clearly showed that the excretion of *p* hydroxyphenylpyruvic acid and the other tyrosine metabolites increased proportionately to increases in dietary phenylalanine and tyrosine The pattern of urinary metabolites found after placing the patient on various dietary and experimental feeding regimens is given in Table 11 1

161 King C G Discussion of paper by G A Jervis (ref 12)
162 Følling A and Closs K Über das Vorkommen von L-Phenylalanin in Harn und Blut bei Imbecillitas phenylpyruvica Ztschr physiol Chem 254 115 1938
163 Waelsch H *Biochemistry of the Developing Nervous System* p 414 Academic Press Inc New York 1955
164 Hsia D Y and Paine R S Phenylketonuria detection of the heterozygous carrier J Ment Deficiency Res 1 53 1957
165 Jervis G A The chemical pathology of disorders due to inborn errors of metabolism in *Third International Neurochemical Symposium* edited by J Folch Pi Pergamon Press Strasburg 1958
166 Knox W E Cullen A M and Rosen B Unpublished results
167 Paine R S and Hsia D Y The dietary phenylalanine requirement and tolerances of phenylketonuric patients A M A Am J Dis Child 94 224 1957
168 Murphy D The dietetic treatment of phenylketonuria Irish J M Sc 391 335 1958
169 Woolf L I Griffiths R and Moncrieff A Treatment of phenylketonuria with a diet low in phenylalanine Brit J Med 1 57 1955
170 Lang K Knopp K and Weber D Treatment of phenylpyruvic oligophrenia Ztschr Kinderh 80 311 1957
171 Horner F A Streamer C W Clader D E Hassel L L Binkley E L and Dumars K W Jr Effect of phenylalanine restricted diet in phenylketonuria II A M A J Dis Child 93 615 1957
172 Horner F A and Streamer C W Phenylketonuria treated from earliest infancy A M A J Dis Child 97 345 1959
173 Brimblecombe F S W Stoneman M and Maliphant R Dietary treatment of an infant with phenylketonuria Lancet 1 609 1959
174 Brimblecombe F S W and Stoneman M E R The treatment of phenylketonuria Proc Roy Soc Med 51 45 1958
175 Gruttner K Muller F and Wallis H Evaluation of success of diet treatment with casein hydrolysate low in phenylalanine in phenylpyruvic oligophrenia Wchnschr Kinderh 106 41 1958

It should be noted that *p*-hydroxyphenylpyruvic acid was excreted continually even during fasting or on a diet of very low tyrosine content Under both these conditions about 1 6 gm of the α keto acid was excreted per day Medes pointed out that this is a reasonable figure for the amount expected from tyrosine derived from the catabolism of endogenous protein and she concluded that the metabolic defect was essentially complete When the patient was placed on a regular diet the amount of urinary *p* hydroxyphenylpyruvic acid doubled and tyrosine could then also be isolated from the urine At still higher levels of tyrosine intake *p*-hydroxyphenyllactic acid was also excreted

One of the most remarkable findings in the studies with this patient was the observation that feeding large amounts of tyrosine al o led to the excretion of 3 4-dihydroxyphenylalanine (dopa) In fact the identification of dopa in the urine of this patient has been cited as one of the most convincing pieces of evidence that dopa is a normal metabolic product of phenylalanine and tyrosine in the higher animals [7] It is assumed that in tyrosinosis the main pathway for tyrosine metabolism is blocked and therefore much more of this amino acid must be metabolized by alternative pathways than under normal conditions

On the basis of the metabolites excreted in the urine of this patient and in order to empha ize the excretion of *p*-hydroxyphenylpyruvic acid perhaps a more appropriate name for this condition would be tyrosyluria [1] One might refer to the case described by Medes as one of *essential* or *idiopathic tyrosyluria* to distinguish it from a number of other conditions in which tyrosyluria has been observed such as scurvy [8-10] pernicious anemia [11] and a variety of other diseases [12]

Spontaneous excretion of *p*-hydroxyphenylpyruvic acid was recently reported by Felix Leonhardi and Glasenapp [13] in two patients with liver disease and a previous splenectomy In these patients however the amount of the α keto acid excreted was regularly less than 50 mg per day and the amount exceeded this value only slightly when extra tyrosine was fed Although the excretion of the α keto acid in these patients is of considerable theoretic interest the amount excreted is so much less than that found in the patient studied by Medes that it is more likely a different type of metabolic abnormality and therefore should not be classed as an additional case of tyrosinosis [1]

On the basis of the pattern of the urinary metabolites found in the urine of this patient Medes proposed that the metabolic defect in tyrosinosis was a failure of the enzymatic step in which *p* hydroxyphenylpyruvic acid is oxidized to homogentisic acid It was believed that the excretion of *p* hydroxyphenylpyruvic acid represented an inability to metabolize this α keto acid and that as a consequence of this defect the earlier steps were also partially impaired thus leading to the excretion of the closely related compounds when extra tyrosine was fed The demon

TABLE 11 1 METABOLIC STUDIES WITH TYROSINOSIS PATIENT

| Diet and supplement | Compounds excreted in the urine | | | |
|---|---|---|---|---|
| | *p-Hydroxyphenyl pyruvic acid gm/day* | *Tyrosine gm/day* | *p-Hydroxyphenyl lactic acid gm/day* | *3 4 Dihydroxyphenyl alanine* |
| Fasting | 1 54 | 0 | 0 | 0 |
| Tyrosine-free diet | 1 64 1 43 | 0 | 0 | 0 |
| Mixed hospital diet | 2 8 | 0 38 | 0 | 0 |
| High protein diet | 3 2 | 0 80 | Trace | 0 |
| High protein diet + 15 gm L-tyrosine | 4 64 | 2 17 | 0 32 | + Urine turned black with alkali |
| High protein diet + 15 gm L-tyrosine | 5 17 | 2 52 | 0 73 | + 30 mg dopa isolated |
| Tyrosine-free diet + 2 gm *p*-hydroxyphenyllactic acid | No increase | 0 | 1 78(4 days) | 0 |
| Tyrosine free diet + 4 gm *p*-hydroxyphenylpyruvic acid on 2 consecutive days | Immediate increase continued for several days | 0 | Increase after 2 days for several days | 0 |
| Tyrosine free diet + 2 gm L-3 4-dihydroxyphenylalanine | Delayed increase maximum on 4th day | 0 100† | 0 | Nearly 0 800 gm recovered *(incomplete)* |

The unusual 2-day delay before an increase in p hydroxyphenylpyruvic acid was found and the rapid rate at which dopa is known to be metabolized [6] suggest that this increase may have been due to variation in the dietary intake of tyrosine rather than the test substance

† It was suggested that the oxidation of tyrosine to dopa is reversible since some tyrosine was excreted after feeding dopa but this is not supported by more recent studies [3] Dehydroxylation of dopa to meta tyrosine has been reported in rabbits [14] but not in man [illegible]

SOURCE G Medes [4]

can be increased by the administration of steroids [23] or by inducing stress conditions [18]

The next enzyme, p-hydroxyphenylpyruvic acid oxidase, is the one proposed by Medes as defective in tyrosinosis. It catalyzes the oxidation

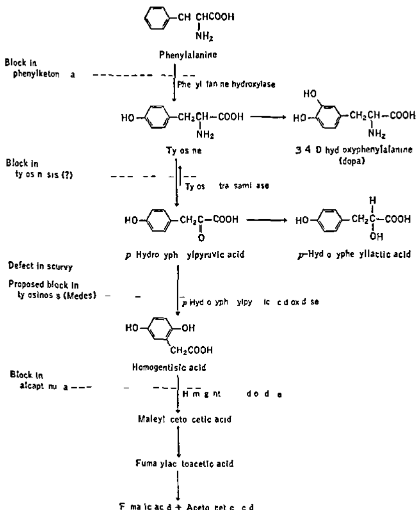



Fig 11 1 Metabolic blocks in the oxidation of phenylalanine and tyrosine

of p-hydroxyphenylpyruvic acid to homogentisic acid and is present in the liver of various mammalian species including hog, rabbit, rat, dog and man. It has also been detected though with lower activity in the kidney of some if not all of these species. Purified p-hydroxyphenyl

stration that administered homogentisic acid was metabolized completely, even when given in relatively large amounts indicated that the defect must be located earlier in the pathway of tyrosine metabolism than it is in alcaptonuria (see Chap 12)

Even though the general scheme of tyrosine metabolism has undergone relatively little modification during the past 30 years the component enzymatic steps have been isolated and intensively investigated within this time Therefore, it is of interest to look again at the evidence upon which these interpretations were made 30 years ago and see whether in view of present understanding of the metabolic pathway the same conclusions would be reached today

## BIOCHEMICAL ASPECTS

Progress in understanding the metabolism of phenylalanine and tyrosine particularly during the past 10 years has been largely due to the successful isolation and purification of the enzymes catalyzing each of the reactions The enzymatic steps pertinent to this discussion are summarized in Fig 11 1 Since several excellent reviews have recently appeared which summarize the numerous papers concerned with these reactions [*1 8 9, 15–17*] only a few points will be mentioned here It is clear that most of the phenylalanine ingested is catabolized via its oxidation to tyrosine and that this hydroxylation step is essentially irreversible For this reason experiments carried out with administered phenylalanine should be equivalent to those in which tyrosine is given if the defect under study involves one of the steps in tyrosine metabolism

The main pathway for tyrosine metabolism in mammals is indicated in Fig 11-1 The first step a transamination reaction with α ketoglutarate to yield p hydroxyphenylpyruvic acid appears to be the rate limiting step of the tyrosine oxidation pathway [*18 19*] At present, it is believed that most of the *p*-hydroxyphenylpyruvic acid derived from tyrosine is formed via transamination and that relatively little of the α keto acid results from oxidative deamination of the amino acid The transamination reaction is catalyzed by a specific transaminase which has been purified by Canellakis and Cohen [*20 21*] from dog liver and more recently by Kenney [*22*] from rat liver Pyridoxal phosphate is the coenzyme and neither pyruvate nor oxaloacetate can replace the requirement for α ketoglutarate The enzyme is specific for L-tyrosine in that no activity is found with phenylalanine tryptophan histidine or a number of other amino acids The transaminase is strongly inhibited by sulfhydryl reagents such as iodoacetate and *p* chloromercuriphenyl sulfonate but it is not inhibited by metal binding agents such as α α dipyridyl diethyldithiocarbamate and 8 hydroxyquinoline Recently evidence has been obtained that the transaminase activity in rat liver

most of the tyrosine is degraded via p hydroxyphenylpyruvic acid to homogentisic acid

## METABOLIC DEFECT IN TYROSINOSIS

Medes proposed [4] on the basis of the tyrosine metabolites found in the urine that the defect in tyrosinosis must be the lack of p hydroxyphenylpyruvic acid oxidase activity The constant excretion of the α keto acid accompanied by closely related compounds when tyrosine was fed suggested that the primary difficulty was in metabolizing this acid and that as a consequence the preceding steps were secondarily impaired

It is of interest to reexamine the balance study data from the tyrosinosis patient in view of current understanding of tyrosine metabolism and to speculate as to whether the same conclusions would be drawn today There is no reason to doubt that the metabolic defect occurs early in the scheme of tyrosine metabolism There are however several observations not in accord with the localization of the defect at the p-hydroxyphenylpyruvic acid oxidase step These are the finding that the predominant metabolite was p hydroxyphenylpyruvic acid with very little p hydroxyphenyllactic acid the apparent completeness of the block at low levels of tyrosine intake yet its incompleteness at moderate or high levels of tyrosine intake and the excretion of dopa when tyrosine was fed

There is a striking difference in the tyrosyluria found in the patient with tyrosinosis from that present in other conditions in which the excretion of p-hydroxyphenylpyruvic acid has been observed In tyrosinosis *only p hydroxyphenylpyruvic* acid was excreted (except on a diet high in tyrosine) whereas in the other conditions *p-hydroxyphenyllactic* acid was a major tyrosyl product in the urine For example premature infants fed extra tyrosine [*10*] were found to excrete several times as much p hydroxyphenyllactic acid as p hydroxyphenylpyruvic acid Scorbutic guinea pigs [*35*] and children [*36*] also excrete appreciable quantities of the lactic derivative [1] This difference in the tyrosyluria was not due to an inability of the tyrosinosis patient to reduce the α keto acid since he excreted a small amount of p hydroxyphenyllactic acid when the diet contained large amounts of tyrosine (Table 11 1) Furthermore when the patient was given *p-hydroxyphenylpyruvic acid* a considerable portion of it was excreted as p-hydroxyphenyllactic acid The latter acid was isolated from the urine and found to be optically active and it

[1] The suggestion has been made [9 , ] that the patient with tyrosinosis may have had unrecognized vitamin C deficiency but the difference in the p-hydroxyphenyllacturia in this condition from that found in scorbutic individuals would seem to exclude this possibility

pyruvic acid oxidase has been prepared from beef [24] pig [24, 25] and dog liver [26–28], and properties of the purified enzyme have been described

The conversion of p hydroxyphenylpyruvic acid to homogentisic acid requires two atoms of oxygen and the liberation of one molecule of $CO_2$. The reaction is complex, it involves hydroxylation of the aromatic ring migration of the side chain, and oxidation and decarboxylation of the pyruvate to an acetate side chain Although it has long been considered *probable that 2,5 dihydroxyphenylpyruvic acid is an intermediate in* this oxidation recent studies with p hydroxyphenylpyruvic acid oxidase preparations from several animal sources [24 26] have shown that 2 5-dihydroxyphenylpyruvic acid is not oxidized to homogentisic acid Furthermore incubation of 2 5 dihydroxyphenylpyruvic acid with normal human liver homogenate (or liver homogenate from an alcaptonuric person) does not yield homogentisic acid [19] The observation made by Neubauer [29] that feeding 2 5-dihydroxyphenylpyruvic acid led to extra homogentisic acid production in an alcaptonuric patient is not inconsistent with these observations if one assumes that even though free 2 5 dihydroxyphenylpyruvic acid is not an intermediate in tyrosine metabolism this compound may be converted to homogentisic acid by some other mechanism

p Hydroxyphenylpyruvic acid oxidase is highly specific for its substrate and does not catalyze the oxidation of other hydroxyphenylpyruvic acids or any of a number of related compounds [24 26] The enzyme requires either ascorbic acid or one of the group of compounds which can replace the vitamin such as the reduced form of 2 6-dichlorophenol indophenol in vitro [15] These agents are required in considerably less than stoichiometric amounts and appear to function by preventing a gradual inhibition of the enzyme by its substrate which would occur in the absence of the protective agent [26–28] Ascorbic acid seems to act in this protective capacity in vivo [30] Although it was proposed several years ago that ascorbic acid acted as a coenzyme for p-hydroxyphenyl pyruvic acid oxidase [31 32] its function in this reaction now seems to be much less specific [15 33 34] and unlike the coenzyme functions as o ciated *with the members of the B vitamins*

The enzyme can be inhibited by relatively low concentrations of diethyldithiocarbamate [26] and by sulfhydryl binding agents such as p-chloromercuribenzoic acid [24] In view of the known affinity of diethyl dithiocarbamate for copper a search has been made for this metal in the purified enzyme but there is no evidence that it is a copper containing protein [24 26]

The occurrence of homogentisic acid as a normal metabolite was subject to some dispute in the past but there is now general agreement that

tyrosine but not of p-hydroxyphenylpyruvic acid The α keto acid rather than tyrosine or p hydroxyphenyllactic acid would be the expected main metabolite in the urine just as was found In addition an elevated tissue level of tyrosine would be more likely to lead to extra dopa being synthesized than would a block in the oxidation of p hydroxyphenyl pyruvic acid If the keto acid which was excreted arose by the metabolism of tyrosine within the kidney (analogous to the keto acid from phenyl alanine in phenylketonuria) it would not be surprising to find this mechanism saturated at moderate levels of tyrosine intake This is in accord with the apparent incompleteness of the block when high dosages of tyrosine were given to the patient

If another patient with tyrosinosis should ever be found it would be particularly important to determine the plasma levels of tyrosine and p-hydroxyphenylpyruvic acid Fortunately a number of microenzymatic methods are now available for these determinations [42 43] The ultimate proof that a particular enzyme is missing in tyrosinosis will of course depend upon the availability of suitable tissues from a patient with this disease

## SUMMARY

1 Tyrosinosis a rare metabolic disease of tyrosine metabolism is characterized by the excretion of p hydroxyphenylpyruvic acid Other *tyrosyl metabolites are also excreted when large amounts of this amino* acid are given Only one case of the disease has ever been found and the inheritance of the condition has not been demonstrated

2 It is generally assumed that the enzyme system defective in tyrosinosis is p hydroxyphenylpyruvic acid oxidase However the defect may well be in the preceding step tyrosine transaminase Evidence supporting the localization of the defect in each of these steps is discussed

## BIBLIOGRAPHY

1 Kretchmer N and Etzwiler D D Disorders associated with the metabolism of phenylalanine and tyrosine Pediatrics 21 445 1958
2 Winchester A M *Genetics* 2d ed p 240 Houghton Mifflin Boston 1958
3 Medes G Berglund H and Lohmann A An unknown reducing urinary substance in myasthenia gravis Proc Soc Exper Biol & Med 25 210 1927
4 Medes G A new error of tyrosine metabolism tyrosinosis The intermediary metabolism of tyrosine and phenylalanine Biochem J 26 917 1932
5 Blatherwick N R Tyrosinosis a search for additional cases J A M A 103 1933 1934
6 Axelrod J D The metabolism of adrenalin and other sympathomimetic amines Physiol Rev 39 51 1959
7 Shaw K N F McMillan A and Armstrong M D The metabolism of 3 4-dihydroxyphenylalanine J Biol Chem 226 55 1957
8 Dalgliesh C E Metabolism of the aromatic amino acids *Advances Protein* Chem 10 31 1955

was presumed to arise by the enzymatic reduction of p hydroxyphenyl pyruvic acid Meister [37] has since reported that p hydroxyphenyl pyruvic acid is active as a substrate with the lactic acid dehydrogenase of muscle

*On the basis of these results one would expect that if p hydroxyphenyl* pyruvic acid were circulating in the tissues of the tyrosinosis patient much more reduction and excretion of the lactic acid analogue would take place It is difficult to understand why only the $\alpha$ keto acid was excreted if the defect were the lack of liver p hydroxyphenylpyruvic acid oxidase activity

On the other hand the urinary metabolites found in the urine in premature infants and in scorbutic guinea pigs and man are those one would expect to find with an impaired activity of liver p hydroxyphenyl pyruvic acid oxidase Evidence that this enzyme is relatively deficient in premature infants has been obtained by Kretchmer et al [38 39] It has also been recently shown that about one half of liver p hydroxy phenylpyruvic acid oxidase activity is inhibited by the administration of p hydroxyphenylpyruvic acid to scorbutic guinea pigs Under these conditions the activities of tyrosine transaminase and homogentisic acid oxidase are not affected [30]

Attempts have been made to induce experimental tyrosinosis in guinea pigs by inhibiting p-hydroxyphenylpyruvic acid oxidase with diethyl dithiocarbamate [40] The administration of this compound results in almost complete inhibition of the oxidase in vivo Animals so treated were injected with p hydroxyphenylpyruvic acid intraperitoneally and even under these conditions very little of the $\alpha$ keto acid was excreted unchanged The main tyrosyl product in the urine was p hydroxy phenyllactic acid [41]

It appears therefore that in the various conditions in which liver p hydroxyphenylpyruvic acid oxidase is known to be impaired one finds a relatively large amount of p hydroxyphenyllactic acid in addition to p hydroxyphenylpyruvic acid in the urine

In view of these observations the different pattern of tyrosyluria found in the patient with tyrosinosis raises the possibility that this metabolic disorder may not be due to a defect in p hydroxyphenylpyruvic *acid oxidase* It also seems unlikely that p hydroxyphenylpyruvic acid was circulating in the tissues except when the intake of tyrosine was very high since the ability to reduce the $\alpha$ keto acid to p hydroxyphenyl lactic acid was demonstrated to be present in this patient (Table 11 1)

One possibility is that the metabolic error in tyrosinosis is much like that found in phenylketonuria with the primary defect a failure to metabolize the amino acid even though the corresponding $\alpha$ keto acid is the main abnormal urinary metabolite If tyrosinosis were analogous to phenylketonuria one would expect to find a high level of circulating

35 Sealock R R and Silberstein H E The excretion of homogentisic acid and other tyrosine metabolites by the vitamin C-deficient guinea pig J Biol Chem 135 251 1940
36 Hulman T H J and Jonxis J H P Some investigations on the metabolism of phenylalanine and tyrosine in children with vitamin C deficiency Arch Dis Childhood 32 77 1957
37 Meister A Reduction of α γ-diketo and α keto acids catalyzed by muscle preparations and by crystalline lactic dehydrogenase J Biol Chem 184 117 1950
38 Kretchmer N Levine S Z McNamara H and Barnett H L Certain aspects of tyrosine metabolism in the young I The development of the tyrosine oxidizing system in human liver J Clin Invest 35 236 1956
39 Kretchmer N Levine S Z and McNamara H The *in vitro* metabolism of tyrosine and its intermediates in the liver of the premature infant A M A J Dis Child 93 19 1957
40 La Du B N and Zannoni V G Tyrosinosis in guinea pigs induced by diethyl dithiocarbamate Fed Proc 17 260 1958
41 La Du B N and Zannoni V G Unpublished observation
42 Knox W E and Pitt B M Enzymic catalysis of the keto-enol tautomerization of phenylpyruvic acids J Biol Chem 225 675 1957
43 La Du B N and Michael P J An enzymatic spectrophotometric method for the determination of phenylalanine in the blood J Lab Clin Med 55 491 1960
44 White A Protein metabolism in *Diseases of Metabolism* 3d ed edited by G G Duncan p 170 Saunders Philadelphia 1952

9 Lerner A B Metabolism of phenylalanine and tyrosine Advances Enzymol **14** 73 1953
10 Levine S Z Tyrosine and phenylalanine metabolism in infants and the role of vitamin C Harvey Lect series **42** 303, 1947
11 Swendseid M L Wandruff B and Bethell F H Urinary phenols in pernicious anemia J Lab Clin Med, **32** 1242 1947
12 Gros H and Kirnberger E J Spontanausscheidung von *p*-Oxyphenylbrenztraubensäure im Harn Klin Wchnschr **32** 115 1954
13 Felix K Leonhardi G and Glasenapp I Über Tyrosinosis Ztschr physiol Chem **287** 141 1951
14 DeEds F Booth A N and Jones F T Methylation and dehydroxylation of phenolic compounds by rats and rabbits J Biol Chem **225** 615 1957
15 Knox W E The metabolism of phenylalanine and tyrosine in *A Symposium on Amino Acid Metabolism* edited by W D McElroy and H B Glass Johns Hopkins Press Baltimore 1955
16 Mason H S Mechanisms of oxygen metabolism Advances Enzymol, **19** 1957
17 Meister A *Biochemistry of the Amino Acids* Academic Press Inc New York 1957
18 Lin E C C and Knox W E Specificity of the adaptive response of tyrosine *α* ketoglutarate transaminase in the rat J Biol Chem **233** 1186 1958
19 La Du B N Zannoni V G Laster L and Seegmiller J L The nature of the defect in tyrosine metabolism in alcaptonuria J Biol Chem **230** 251 1958
20 Canellakis Z N and Cohen P P Purification studies of tyrosine-*α* ketoglutaric acid transaminase J Biol Chem **222** 53 1956
21 Canellakis Z N and Cohen P P Kinetic and substrate specificity study of tyrosine *α* ketoglutaric acid transaminase J Biol Chem **222** 63 1956
22 Kenney F T Properties of partially purified tyrosine *α* ketoglutarate transaminase from rat liver J Biol Chem **234** 2707 1959
23 Lin E C C and Knox W E Adaptation of the rat liver tyrosine *α* ketoglutarate transaminase Biochim et biophys acta **26** 85 1957
24 Hager S E Gregerman R I and Knox W E *p*-Hydroxyphenylpyruvate oxidase of liver J Biol Chem **225** 935 1957
25 Roka L Konig G and Rubner H Die fermentative Oxydation von *p*-Hydroxyphenylbrenztraubensäure zu Homogentisinsäure Ztschr physiol Chem **313** 87 1958
26 La Du B N and Zannoni V G The tyrosine oxidation system of liver II Oxidation of *p*-hydroxyphenylpyruvic acid to homogentisic acid J Biol Chem **217** 777 1955
27 La Du B N and Zannoni V G The tyrosine oxidation system of liver III Further studies on the oxidation of *p* hydroxyphenylpyruvic acid J Biol Chem **219** 273 1956
28 Zannoni V G and La Du B N The tyrosine oxidation system of liver IV Studies on the inhibition of *p*-hydroxyphenylpyruvic acid oxidase by excess substrate J Biol Chem **234** 2925 1959
29 Neubauer O Über den Abbau der Aminosäuren im gesunden und kranken Organismus Deutsches Arch klin Med **95** 211 1909
30 Zannoni V G and La Du B N Studies on the defect in tyrosine metabolism in scorbutic guinea pigs J Biol Chem **235** 165 1960
31 Sealock R R and Goodland R L Ascorbic acid a coenzyme in tyrosine oxidation Science **114** 645 1951
32 Sealock R R Goodland R L Sumerwell W N and Brierly J M The role of ascorbic acid in the oxidation of L-tyrosine by guinea pig liver extracts J Biol Chem **196** 761 1952
33 La Du B N Jr and Greenberg D M Ascorbic acid and the oxidation of tyrosine Science **117** 111 1953
34 Knox W E Coenzyme functions of ascorbic acid Symposium on Vitamin Metabolism in *Proceedings of the IVth International Congress of Biochemistry* Vienna 1958 vol XI Pergamon Press Ltd London

Boedeker precipitated homogentisic acid from alcaptonuric urine as the lead salt [2 3] but he was unable to obtain enough material to purify it and determine its exact chemical structure He did point out the similarity of its behavior in alkali with that of known hydroxyphenols

*During the following 30 years there was considerable confusion as to* the chemical structure of "alkapton It was variously reported by different groups to be catechol [4 5] protocatechuic acid [6] "glycosuric acid [7] and uroleucic acid [8] The various claims and disputes about its composition during this period are well summarized in a recent review by Knox [9] The controversy ended and the chemical structure of alcapton was firmly established in 1891 by the excellent work of Wolkow and Baumann [10] They identified it as 2 5-dihydroxyphenyl acetic acid and named it *homogentisic acid* because of its close structural relationship to gentisic acid (2 5-dihydroxybenzoic acid) The earlier reports of other substances such as uroleucic acid in alcaptonuric urine were shown to be the result of analytical errors from contamination with various normal urinary constituents [11]

Once the aromatic structure of homogentisic acid was known it was not long before various suggestions were made as to the source of this unusual urinary product The known aromatic substances in proteins tyrosine and phenylalanine were of course the primary suspects (Fig 12 1) Wolkow and Baumann demonstrated in 1891 [10] that feeding

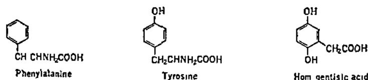



Fig 12-1 Formulas of phenylalanine tyrosine and homogentisic acid

either extra tyrosine or a diet high in protein greatly increased the amount of homogentisic acid excreted by an alcaptonuric patient Even though they were incorrect in believing that the formation of homogentisic acid was due to the activity of bacteria in the gut their important observations initiated a number of studies by clinical investigators of that time who used the metabolic defect in alcaptonuric individuals to determine the pathway by which phenylalanine and tyrosine are metabolized to homogentisic acid Numerous compounds which were possible

alkali and the Greek root ἅπτω meaning to seize to possess the combination therefore meaning to have an affinity for alkali *The Oxford English Dictionary* [165] gives the correct origin and indicates that the k of alkaptonuria comes from the Greek root ἅπτω rather than from alkali Transliteration of the k to c therefore follows the same pattern used in many words derived from similar Greek roots

# Chapter 12

# Alcaptonuria

*Bert N La Du*

Alcaptonuria is a rare hereditary metabolic disease in which the enzyme homogentisic acid oxidase is missing Because of this defect, homogentisic acid produced during the metabolism of phenylalanine and tyrosine cannot be further metabolized it therefore accumulates and is excreted in the urine

If urine containing homogentisic acid is allowed to stand for some time, it gradually turns dark as the acid is oxidized to a melanin like product The polymerization is speeded by alkali this explains why washing diapers of alcaptonuric infants with soap tends to make the stains more intense instead of removing them

It is not surprising that such an obvious sign as dark urine led to the early recognition of this disease Several persons reported in the medical literature of the sixteenth and seventeenth centuries who continually passed dark urine are presumed to have had alcaptonuria—see Garrod [*1*] The first patient in whom the diagnosis was made with certainty was one described by Boedeker in 1859 [*2*] He recognized that the reducing properties of this patient s urine were different from those of one containing glucose (for example it did not reduce bismuth hydroxide) and he observed the darkening of the urine when alkali was added He used the property of avid oxygen uptake in alkaline solution to give the substance a name '*in alkalischer Losung bei gewohnlicher Temperatur den Sauerstoff begierig zu verschlucken und nannte ihn danach Alcapton (freilich recht barbarisch zusammengesetzt aus dem arabischen* alkali *und dem griechischen* κάπτειν *begierig verschlucken* ) —[*2* p 139] Two year later [*3*] Boedeker spelled it ' Alkapton and since then this condition has been known as *Alkaptonurie* in the German literature and as *alcaptonurie* in the French Both c and k have been used by writers in English [1]

[1] Several medical dictionaries erroneously assume alkapton to be derived from

metabolism in 1909 [12] the first such scheme for any of the amino acids In 1928 [13] he revised it to incorporate the results obtained during the intervening years (Fig 12 2) Although a few changes have been made since then the basic scheme which he postulated has remained essentially unchanged during the last 30 years

Studies on alcaptonuria have been of general importance in the development of ideas about diseases of metabolism As a result of his studies on alcaptonuria Sir Archibald Garrod developed his whole concept of inheritable metabolic diseases In 1908 he discussed alcaptonuria in one of the Croonian Lectures [1] and in the following year he expanded his ideas more completely in his classic book *Inborn Errors of Metabolism* [11] He thought of alcaptonuria as a metabolic "freak or 'sport comparable to a structural abnormality rather than as a disease in the usual sense He felt that patterns of metabolism varied in each individual according to his hereditary background and that alcaptonuria and the other inborn errors of metabolism represented extreme examples of such variant possibilities [14] He suspected that these variations ultimately might depend upon differences in the activity of specific enzymes thus anticipating by many years the conclusion of Beadle and Tatum [15] that a single defective gene is correlated with a metabolic block in one enzymatic reaction

In 1909 Garrod [11] wrote of the probable defect in alcaptonuria

> We may further conceive that the splitting of the benzene ring in normal metabolism is the work of a special enzyme that in congenital alcaptonuria this enzyme is wanting whilst in disease its working may be partially or even completely inhibited The experiments of G Embden and others upon perfusion of the liver suggest that organ as the most probable seat of the change

Garrod s supposition that a specific enzyme is missing in alcaptonuria has been supported through the years by many types of circumstantial evidence and recently has been confirmed by direct biochemical assay of alcaptonuric liver preparations [16]

## CLINICAL FEATURES

The cardinal features of alcaptonuria are signs due to the presence of homogentisic acid in the urine pigmentation of cartilage and other connective tissues and nearly always in later years arthritis

### URINARY CHANGES

According to the usual textbook description people with alcaptonuria give a history of dark urine or urine which turns dark on standing It should be emphasized that in a large number of alcaptonuric patients this finding is not observed Many patients have never noted any ab-

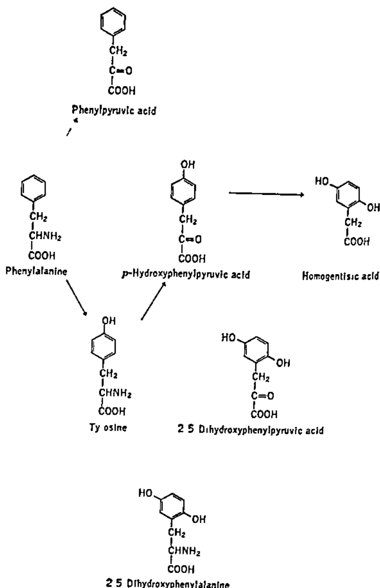



Fig 1? 2 Scheme of phenylalanine and tyrosine metabolism to homogentisic acid based upon feeding experiments with alcaptonuric patients [13] The dotted arrows show various pathways considered possible by Neubauer the solid arrows indicate the pathway as it is viewed today

intermediary substances in the formation of homogentisic acid were fed to alcaptonuric patients It was expected that compounds in the metabolic sequence would increase the excretion of homogentisic acid but that those which were not intermediates would fail to do so On the basis of such studies Neubauer suggested a preliminary scheme of tyrosine

isolated from the urine after precipitation as the lead salt [*27, 31*] and the product shown to have the correct chemical composition and melting point Paper chromatography of the urine directly or of the product obtained by extracting acidified urine with ether, furnishes a simple technique to identify homogentisic acid [*32*] More recently a specific enzymatic method has been developed which permits the quantitative analysis of homogentisic acid in urine blood and other tissues [*33, 34*]

## OCHRONOSIS

In 1866 Virchow described a peculiar type of generalized pigmentation in the connective tissues of a 67 year-old man [*35*] The pigment was gray to bluish black grossly but an ochre color microscopically and for this reason he named the condition *ochronosis* Although the patient s clinical history is not known it is quite certain that Virchow described for the first time the generalized pigmentation which gradually develops in alcaptonuria Actually it was not until nearly 40 years later that Albrecht in 1902 [36] clearly demonstrated the connection between ochronosis and alcaptonuria Not long after Osler diagnosed ochronosis clinically for the first time in two alcaptonuric brothers [*37*] He recog nized that the pigmentation of the sclerae and ears were signs of the same metabolic abnormality that had previously been detected only by changes in the urine Perhaps it is not unexpected that there was such a delay in the clinical recognition of the ochronotic pigmentation in alcaptonuria Generally the earliest change that can be detected externally is a slight pigmentation of the sclerae or the ears but these changes are rarely noticeable before the alcaptonuric patient is 20 or 30 years old The eye pigmentation is usually found about midway between the cornea and outer and inner canthi at the site of the insertions of the recti muscles (Fig 12 3) In addition a more diffuse pigmentation may also involve the conjunctiva and cornea [*39*] The typical pigmentary changes in the ear cartilages similarly occur only in long-standing alcaptonuria The cartilage is slate blue or gray and feels irregular and thickened It is first seen in the concha and the antihelix and later in the tragus It is sometimes reported that a dusky discoloration corresponding to the underlying tendons can be seen through the skin over the hands The prominence of this pigmentation is variable and in many instances it is scarcely evident at all The pigment appears in perspiration clothing near the axillary regions may be stained and the skin may have a brown i h discoloration in the axillary and genital regions [*24*]

In contrast to these rather minimal findings the pigmentation ob served in the tissues of an elderly alcaptonuric patient at operation or at post mortem is indeed striking [*35, 40–42*] Cartilage in many areas particularly the costal laryngeal and tracheal cartilage is densely pig mented and is described as being coal black in some areas Pigmentation

normality in the color of the urine during childhood [17–19] and diagnosis has been made only after they sought treatment for arthritis during their later years [20–23]. In some cases diagnosis has followed a false-positive test for diabetes [20, 22] or the finding of the unusual and distinctive x-ray changes in the spine [24]. In others the disease has not been suspected until a surgical procedure has revealed marked pigmentation of the cartilage [25].

Alcaptonuric individuals on a normal diet void a urine which at first is not an abnormal color and which does not darken for many hours if *it remains at an acid pH. This is true even for patients with extensive* ochronosis. It appears, therefore, that in those instances where freshly voided urine turns dark quickly, additional factors must be involved. Two factors that would favor rapid darkening are the excretion of an alkaline urine and a lower concentration than normal of vitamin C and possibly other reducing agents usually present in the urine. It is well known that vitamin C protects homogentisic acid against oxidation, and in the past vitamin C has been suggested as a therapeutic agent because of this property [26].

The unusual findings in alcaptonuric urine can all be attributed to one abnormal constituent, homogentisic acid. No abnormal amino acid pattern [17] or other tyrosine metabolic products are found [27].[2] The various diagnostic tests for alcaptonuria by urinalysis, therefore, are all based upon the detection of homogentisic acid through its unusual chemical properties. Its ease of oxidation results in a gradual darkening of the urine downward from the surface until the entire sample is dark brown; this darkening is greatly accelerated by alkali. Further evidence of its ease of oxidation is the behavior of alcaptonuric urine in its reaction with Benedict's sugar reagent. Homogentisic acid not only reduces the copper reagent to yield a yellow-orange precipitate but it also undergoes darkening because of the alkalinity of the reagent. The net effect is an orange precipitate in a muddy brown solution. The reduction of molybdate is the basis of the Briggs's test, commonly used to follow the urinary excretion of homogentisic acid [28]. Reduction of silver in photographic paper emulsion has been used as a qualitative test [29] and as the basis of a quantitative method to measure this acid [30]. Homogentisic acid *is not fermented by yeast,* and it does not fluoresce under ultraviolet light.

A presumptive diagnosis of alcaptonuria can be made on the basis of the results of these nonspecific tests, but a more specific means for its identification is desirable. In many cases, homogentisic acid has been

[2] It has been reported that an alcaptonuric patient excreting about 7 gm homogentisic acid also excreted about 0.5 mg gentisic acid per day [166]. The conversion of small amounts of homogentisic acid to gentisic acid has been demonstrated in homogenates of rabbit liver [167].

been determined It is possible that some other constituents in addition to homogentisic acid are included in the product just as melanin obviously contains more than a polymerized dopa unit i e, a considerable quantity of sulfur [45]

The formation of the pigment in the tissues may be entirely nonenzymatic like the darkening of alcaptonuric urine Solutions of pure

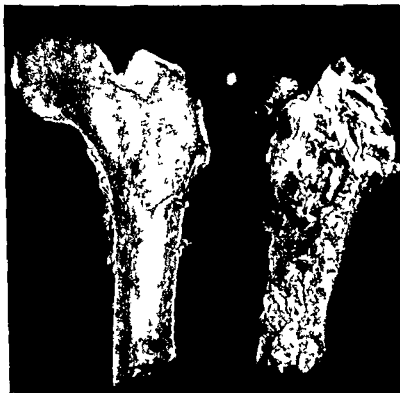


Fig 12-4 Ochronotic pigmentation of the femur of a 56-year-old alcaptonuric subject (*Courtesy of Dr H W Edmonds of the Washington Hospital Center Washington D C*)

homogentisic acid made alkaline and aerated with air or oxygen form a dark brown product which has an ultraviolet absorption peak at 250 mμ—Milch et al [46] Unfortunately the pigment deposited in the tissues of an alcaptonuric patient with ochronosis has not been analyzed in the same way and so the relevance of the model polymerization to the in vivo process has not been established Milch and Titus [47] have recently suggested that since polymerization of pure homogentisic acid solutions does not proceed at neutral pH and low partial pressure of oxygen (as occurs in cartilage) it is likely that if molecular oxygen is

is also present throughout the body in fibrous tissues, fibrocartilage tendons and ligaments (Fig 12-4) To a lesser degree it is also found in the endocardium the intima of the larger vessels in various organs such as kidney and lung and in the epidermis Microscopic examination shows the pigment to be deposited both intercellularly and intracellularly, and it may be either granular or homogeneous Like melanin the ochronotic pigment is bleached when treated for 24 hr with hydrogen peroxide and it is soluble in alkali, but only slightly soluble in hydrochloric acid



Fig 12-3 The bilateral deposition of ochronotic pigment in the sclerae best seen in the left eye [38]

Thus in many of its chemical characteristics the ochronotic pigment resembles melanin arising from 3 4 dihydroxyphenylalanine (dopa) Unfortunately, there is no specific stain to distinguish the ochronotic pigment of alcaptonuria from melanin derived from other sources Although Fitzpatrick [43] states that Becker's silver stain for melanin is not darkened by ochronotic pigment and that the latter is stained intensely black with polychrome methylene blue more recently Cooper and Moran [42] compared the staining properties of ochronotic pigment and melanin employing a number of special stains They concluded that no specific differentiation can be made with any of the stains used Both pigments were best detected by the Nile blue stains of Lillie [44] Variations with trichrome cresyl violet and the periodic acid-Schiff (PAS) stains seemed to depend mainly upon the differences in the amount of pigment present rather than the type of pigment represented

The pigment deposited in ochronosis is presumably a polymer derived from homogentisic acid but its exact chemical structure has not yet

culosis No mention was made of ochronotic changes in their connective tissues but it is unlikely that if they were present to the degree found in most alcaptonuric patients they would have gone unnoticed

## ARTHRITIS

"Ochronotic arthritis is a manifestation of long standing alcaptonuria From the case reports in the literature it appears that alcaptonuric arthritis occurs at an earlier age and is more severe in males than in

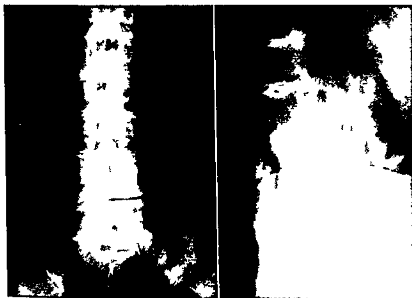


Fig 12 5 Roentgenograms of the spine showing the typical narrowing and calcification of the intervertebral disks [33]

females [61] even though the sex incidence of alcaptonuria is roughly equal This sex difference in ochronotic arthritis is reminiscent of the similar preponderance of gouty arthritis in males Hench [62] has stated that ochronotic arthritis resembles rheumatoid arthritis clinically but osteoarthritis roentgenographically The earliest symptoms observed are usually some degree of limitation of motion of the hip knee joints or occasionally the shoulders There are nearly always periods of acute inflammation which may resemble rheumatoid arthritis and later there is usually rather marked limitation of motion and ankylosis in the lumbosacral region

X rays may reveal changes considered almost pathognomonic of alcaptonuria [24] The vertebral bodies of the lumbar spine show degeneration of the intervertebral disks with a narrowing of the space and dense calcification of the remaining disk material (Fig 12 5) This is

involved in vivo it involves the participation of an enzyme system present locally This conclusion assumes not only that the composition and steps of formation of the ochronotic pigment would be the same in vivo as in solutions of pure homogentisic acid, but that the pigment is both formed and deposited within the cartilage It is not possible today to say to what degree enzymes play a role in the synthesis of the ochronotic pigmentation in alcaptonuria although on the basis of their specificity it seems reasonable to exclude the enzymes involved in the synthesis of melanin via dopa

OCHRONOSIS NOT DUE TO HOMOGENTISIC ACID

Clinically ochronosis due to alcaptonuria might possibly be confused with the pigmentation of the skin, nail beds, conjunctivas and cartilage seen in persons who have taken Atabrine for many months *[48]* Of course the history and the failure to find homogentisic acid in the urine should establish the diagnosis

Another type of acquired ochronosis is that which is secondary to the prolonged use of carbolic acid dressings for chronic cutaneous ulcers *[49-54]* This pigmentation is reversible and recedes after the medication is discontinued Since this agent is rarely used today, nearly all the cases of ochronosis seen now are secondary to alcaptonuria There are however a few puzzling cases of ochronosis in the literature in which exogenous agents such as phenol can probably safely be excluded as causative agents and in which the urine is reported to contain no homogentisic acid *[55 56]* In the case described by Oppenheimer and Kline *[55]* a determined effort to isolate homogentisic acid from the urine was made by Janney *[57]* He concluded that this was an example of ochronosis secondary to a melanuria but no aromatic metabolite related to dopa was identified in the urine He was able to isolate a melanin like pigment from the urine and from a prostatic calculus which had by elementary analysis much the same values as those reported by Morner *[45]* for melanin obtained from the urine and tumor tissue of a patient with a melanosarcoma It is possible that melanotic tumors might cause a generalized ochronosis also but it is unlikely that a patient with such a condition would survive long enough for this complication to become evident *[50]* [2]

On the other hand instances in which alcaptonuria unaccompanied by ochronosis *has been diagnosed with certainty are extremely rare* From the nature and completeness of the metabolic defect one would expect that all alcaptonuric patients would develop ochronosis to some degree if they live to middle age The two patients with alcaptonuria who did not have ochronosis at autopsy *[59 60]* had extensive tuber

[2] In one case of alcaptonuria the ochronotic pigment in the eye was misdiagnosed as a melanosarcoma and the eye was removed *[58]*

nosis and cardiovascular disease is not clearly established but a review of the case histories of alcaptonuric patients indicates that there is a high incidence of heart di case [22] In 1910 Beddard [52] tabulated the autopsy findings in 11 cases of ochronosis (none due to treatment with phenol) and found that 8 had chronic mitral and aortic valvulitis 1 had an aortic aneurysm and 1 an aneurysm of the left ventricle Other investigators [40 65] have noted generalized arteriosclerosis and calcification in the heart valves and of the annulus of the aortic and mitral valves [41] Myocardial infarction is a common cause of death in this group

Other complications reported in alcaptonuric patients are ruptured intervertebral disks [66] and prostatitis [17 22 65 67] or renal stones—ee Young [67] Clinical case reports on new cases of alcaptonuria should be encouraged in order that additional phenomena related to this met abolic di ease may be revealed One case of alcaptonuria with poly cythemia [68] and one with severe renal disease called ochronotic nephrosis [42] remain isolated examples presumably because of chance association with other diseases It should be kept in mind that conditions which favor the expression of alcaptonuria such as consanguineous marriages would also favor the manifestation of other recessive but unrelated traits

## SYNTHESIS AND DEGRADATION OF HOMOGENTISIC ACID

### BIOSYNTHESIS

In mammals most of the dietary phenylalanine and tyrosine is oxidized to acetoacetic acid by enzyme systems localized primarily in the liver and kidney The scheme of this metabolic pathway is shown in Fig 12 6 Several excellent reviews of phenylalanine and tyrosine metabolism have appeared during the past few years [69–72] These may be consulted for the detailed experimental evidence supporting each of the steps in this scheme As mentioned above the scheme is based upon the earlier studies with alcaptonuric patients and many animal experiments in vivo It has been revised and extended particularly during the past 10 years by a large number of experiments on tyrosine metabolism in vitro

In some of these in vitro studies isotopically labeled phenylalanine and tyrosine were used to determine the fate of each of the carbon atoms in the aromatic ring and of the side chain [73–77] The labeled amino acids were incubated with liver slices and the distribution of the i otope was determined in the products CO acetoacetic acid and fumaric acid The e experiments showed that two of the four carbon atoms of aceto-acetic acid were derived from carbon atoms 2 and 3 of the side chain and that the other two came from the ring Furthermore the position of the i otope from the ring carbon atoms indicated that the side chain must have migrated during the oxidation (Fig 12 7) The isotopic evidence

accompanied to a variable degree by fusion of the vertebral bodies From the x ray changes in the lumbar spine alone, it is often possible to be reasonably certain of the diagnosis of alcaptonuria In contrast to rheumatoid spondylitis little osteophyte formation and minimal calcification of the intervertebral ligaments are present The large peripheral joints involved also differ from osteoarthritis in that the degenerative joint changes in ochronotic arthritis are most commonly the shoulder and hip whereas such joints as the sacroiliac may be completely spared

Calcification of the ear cartilage is another sign of the disease that may be observed by x ray The large joints affected generally show degenerative osteoarthritic changes with calcified deposits most commonly in the muscle tendons around the large joints Occasionally free intraarticular bodies are found *[63]* By contrast, the smaller joints usually show little or no abnormality

The common occurrence of arthritis in the general population and the long period before its onset in patients with alcaptonuria no doubt account for the failure of the earlier investigators to appreciate the association of arthritis with alcaptonuria The first case described by Boedeker was reported to have neuralgia of the lower lumbar spine The early investigators considered alcaptonuria a completely benign disease without symptoms and of clinical importance only in that it might be misdiagnosed as diabetes A review of the earlier case reports shows that in most instances osteoarthritis was mentioned and indeed, nearly all alcaptonuric patients develop arthritis during their later years The arthritic complications are often severe and painful and may lead to a completely bedridden existence in later life

The relationship between the deposition of pigment in the connective tissue and the degenerative changes which occur in some areas of the connective tissues particularly the cartilage and the intervertebral disks remains unknown It has been proposed that the pigment acts as a chemical irritant to accelerate a degenerative process in the cartilage leading to changes similar to those in osteoarthritis *[21, 63]* There is no evidence to support this suggestion It is also possible that either the pigment or homogentisic acid itself might inhibit some of the enzyme systems involved in cartilage metabolism Recently Greiling *[64]* has shown that low concentrations of the pigment prepared by treating homogentisic acid with alkali inhibit the action of hyaluronidase on chondroitin sulfuric acid and on hyaluronic acid but homogentisic acid does not act as an inhibitor at the same concentrations

## OTHER FINDINGS IN ALCAPTONURIA

In addition to the features mentioned above other complications seem to occur in alcaptonuric patients with a greater frequency than might be anticipated in the general population The relationship between ochro-

was entirely in agreement with the scheme postulated earlier by Neubauer [13] (Fig 12 2) to account for the 2 5-dihydroxyphenyl intermediary product homogentisic acid from the 4 hydroxyphenyl substrates This rearrangement was believed to involve a quinol intermediate with a migration of the side chain much like the migration of the methyl group in the oxidation of p cresol [78] (Fig 12 8) This unusual migration in the

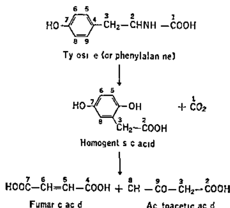



Fig 12 7 Fate of each of the carbon atoms of phenylalanine or tyrosine based upon experiments with the amino acids labeled with isotopic carbon

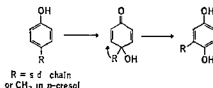



Fig 12 8 Formation of quinol intermediate and migration of the substituent (CH in p-cresol or side chain) in p-hydroxyphenylpyruvic acid oxidation

oxidation of p hydroxyphenylpyruvic acid to homogentisic acid is even today the least understood step of any in the scheme shown in Fig 12 6 So far it has not been possible to identify a free intermediate in this step It appears as though the hydroxylation of the ring and the migration and oxidative decarboxylation of the side chain all take place as a complicated single step This reaction is discussed in more detail in the chapter on tyrosinosis (Chap 11)

## HOMOGENTISIC ACID A NORMAL INTERMEDIATE

The presence of homogentisic acid as a normal intermediate in the scheme deserves further comment It should be recalled that in 1911 Dakin [79] suggested that not only was homogentisic acid an abnormal

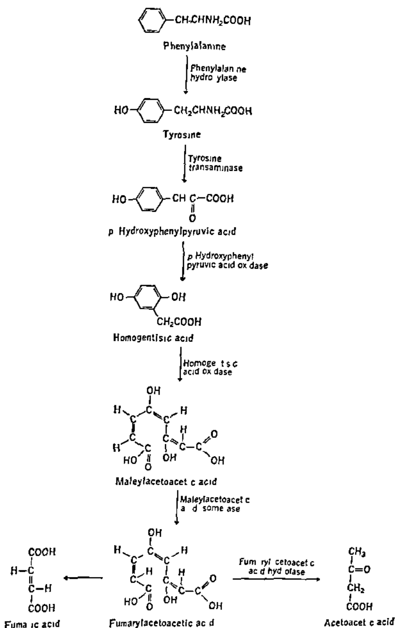



Fig 19 6 Enzymatic steps in the oxidation of phenylalanine and tyrosine to acetoacetic acid

## METABOLIC DEFECT IN ALCAPTONURIA

Even though Garrod suggested in 1908 (*11*) that the metabolic defect in alcaptonuria was the absence of the liver enzyme catalyzing the oxidation of homogentisic acid other possible explanations have been offered from time to time by other workers At the time Garrod presented his theory it was quite generally believed that alcaptonuria was due to the formation of homogentisic acid by intestinal organisms This opinion was based upon an assumption of Wolkow and Baumann [*10*] that the synthesis of homogentisic acid from tyrosine or phenylalanine was too complicated to be accomplished by human tissues and therefore must have resulted from the action of bacteria in the gut

In 1914 Gross [*87*] presented evidence for an enzymatic defect in alcaptonuria He reported that homogentisic acid did not disappear when it was incubated with the serum from an alcaptonuric patient whereas it did disappear when incubated with the serum from normal individuals presumably because of the presence in normal serum of a homogentisic acid–oxidizing enzyme A few years later Katsch and Stern [*88*] suggested that it was not an enzyme that was missing in alcaptonuric serum but that the results of Gross were due to the presence of an inhibitor in alcaptonuric blood Both these findings were disputed by Lanyar and Lieb [*89*] who pointed out that the previous workers had not controlled the experimental conditions satisfactorily particularly the pH and that autooxidation of homogentisic acid probably accounted for their results Unfortunately the report that Gross found an enzyme missing in alcaptonuric blood is still widely quoted in medical texts It is now certain that his results were in error since plasma contains no detectable homogentisic acid oxidase [*33*]

Another proposal as to the nature of the defect was that of Dakin [*79*] as discussed above in which he considered that the whole pathway to homogentisic acid in alcaptonuric patients is abnormal More recently Neuberger [*27*] suggested that perhaps the alcaptonuric kidney differs from the normal kidney in having the capacity to secrete homogentisic acid actively while the normal kidney might not and that this unusual renal defect might account for the abnormal urinary product —see Dent [*135*] However throughout the last 50 years Garrod s suggestion that a liver enzyme is missing has been generally considered the most reasonable hypothesis It was obvious that the net effect would be the same whether the enzyme itself or a vital cofactor is missing or whether there is an inhibitor of the enzyme in alcaptonuric liver

A recent analysis of the enzymes involved in tyrosine metabolism in normal and alcaptonuric liver showed that only homogentisic acid oxidase is missing in alcaptonuric liver and that all the other enzymes involved in tyrosine metabolism to acetoacetic acid are present and have about

urinary product but that its formation resulted from the metabolism of tyrosine by an abnormal pathway in alcaptonuria The main support for this proposal was that when he fed animals and an alcaptonuric patient [80, 81] derivatives of phenylalanine and tyrosine (p-methylphenylalanine and p methoxyphenylalanine) which because of their para substituent, could not form quinol intermediates he found them to be well metabolized (in his view, by the normal pathway of tyrosine metabolism) He also found that these compounds caused an increase in acetoacetic acid when perfused through dog liver, as did phenylalanine and tyrosine Although Dakin s results remained unexplained, through the years more and more evidence has accumulated that homogentisic acid is indeed a normal intermediate For example it was shown that some homogentisic acid accumulated from tyrosine in rat liver homogenates under certain experimental conditions [32 70] more recently, it was shown that homogentisic acid accumulated quantitatively from tyrosine or p hydroxyphenyl pyruvic acid in dog liver [82] and human liver [16] preparations in the presence of $\alpha\,\alpha'$ dipyridyl an inhibitor of homogentisic acid oxidase Homogentisic acid oxidase is widely distributed in nature and where it occurs it is associated with the other enzymes involved in the oxidation of tyrosine to acetoacetic acid

There still remained the problem of explaining Dakin's results In 1957 Lerner, Gottesman and Crandall [83] reinvestigated the ketogenic effect of the tyrosine analogues They found that p methoxy DL-phenyl alanine is not ketogenic and that neither the L-form nor p-methoxyphenyl alanine was metabolized although the D isomer was deaminated in dog liver They attributed the earlier results of Wakeman and Dakin [81] to the ketogenic effect of the ammonium ion released by the deamination of the D-component (The ketogenic effect of ammonium ion [84] was not known in 1911 ) It must also be inferred from these results that Dakin did not have sufficiently sensitive methods to detect the excretion of the unchanged compounds in the urine The present scheme of tyrosine oxidation (Fig 12 6) is therefore very much like that of Neubauer (Fig 12 2) in the steps leading to the formation of homogentisic acid with the notable exception that neither 2 5 dihydroxyphenylalanine nor 2 5-dihydroxyphenylpyruvic acid is now considered a likely intermediate Both these compounds produced extra homogentisic acid when fed to alcaptonuric patients [12 27] but they were found to be inactive as substrates when tested with mammalian liver preparations which oxidize tyrosine or p-hydroxyphenylpyruvic acid to homogentisic acid [16 32 82 85] These results also exclude the alternative pathway suggested by Neuberger in 1947 in which tyrosine would be oxidized first to 2 5-dihydroxyphenylalanine and then either through 2 5-dihydroxyphenyl pyruvic acid or 2 5-dihydroxyphenylethylamine to homogentisic acid [86]

evident 38 hr after birth, although after 52 hr they were deeply stained and continued to be thereafter. The reason for the delay in the excretion of homogentisic acid in newborn alcaptonuric patients is probably that the enzyme systems involved in tyrosine oxidation are not completely developed at birth and that they increase in activity during the next few days [94]. Once established, the defect continues relentlessly throughout life. No therapeutic agent has been found which substantially alters *the degree of the defect. The amount of homogentisic acid excreted per* day is usually from 4 to 8 gm; it can be altered by changing the content of phenylalanine and tyrosine in the diet. In starvation there is a marked decrease in homogentisic acid excretion [95], as would be expected, although Mittlebach [96] showed that on a diet very low in protein the alcaptonuric patient continued to excrete some homogentisic acid, presumably from the breakdown of tissue proteins.

### HOMOGENTISIC ACID OXIDASE

The enzymatic step which is missing in alcaptonuria is the further metabolism of homogentisic acid by an oxidative cleavage of the ring to yield maleylacetoacetic acid [97], which in turn is isomerized enzymatically to fumarylacetoacetic acid [98–100] (Fig 12 6). The next step is hydrolysis to fumaric and acetoacetic acids by an enzyme which appears to be the same as that shown to hydrolyze a number of α,γ-diketo acids by Meister and Greenstein [100a] and to hydrolyze triacetic acid [101, 102].

In 1951 Suda and Takeda [103] solubilized an enzyme from a strain of *Pseudomonas* adapted to tyrosine which catalyzed the oxidation of homogentisic acid; they named it *homogentisicase*. They then studied the properties of a similar enzyme from rabbit liver [104]. Homogentisicase or, as it is more generally called, homogentisic acid oxidase, has been purified to some degree in several laboratories and many of its properties have been described [97, 105–107]. It belongs to the class of oxygenases. In the cleavage of the benzene ring both oxygen atoms come from atmospheric oxygen, as indicated recently in experiments with $O^{18}$ [108]. The enzyme contains essential sulfhydryl groups and requires ferrous iron [104, 109] *as do several of the other oxygenases involved in ring* cleavage reactions such as pyrocatechase [110, 111], hydroxyanthranilate oxidase [112] and protocatechuic acid oxidase [113, 114] (see Mason [115] p 126). No other cofactors have been clearly implicated in this reaction. Although evidence that one did exist was presented by Suda and Takeda [104] in 1950, it now appears that a protective effect of glutathione may account for the earlier results [116]. There is also general agreement that the previously suggested requirement for ascorbic acid in this enzyme system [117] is an indirect one due to the requirement for ferrous iron. The only function that has been demonstrated for ascorbic acid in this reaction is to maintain iron in the reduced form [104, 118].

the same activity as in normal liver [16, 88] (Table 12 1) Evidence was also obtained that the lack of activity is not due to the presence of inhibitor or to the lack of any known cofactor [16] It now seems reasonable to define the defect in alcaptonuria as the failure to synthesize active homogentisic acid oxidase and to attribute all the findings in alcaptonuria to this specific enzymatic defect Whether these individuals form a catalytically inactive protein differing perhaps only slightly in structure from active homogentisic acid oxidase, or whether they produce no protein at all resembling the enzyme is still unknown

TABLE 12 1 ACTIVITY OF TYROSINE OXIDATION ENZYMES IN ALCAPTONURIC AND NONALCAPTONURIC HUMAN LIVER HOMOGENATE

| Enzymes | Enzyme activity µmoles of substrate oxidized/hr/gm of liver | |
|---|---|---|
| | Nonalcaptonuric | Alcaptonuric |
| Tyrosine transaminase | 36 | 32 |
| p-Hydroxyphenylpyruvic acid oxidase | 67 | 46 |
| Homogentisic acid oxidase | 268 | <0 048 |
| Maleylacetoacetic acid isomerase | 960 | 780 |
| Fumarylacetoacetic acid hydrolase | 288 | 222 |

Units calculated as Δ log optical density per hour per 0 1 gm wet weight of liver [98]
SOURCE B N La Du et al [16]

A recent opportunity to obtain at autopsy samples of kidney tissue from an alcaptonuric patient made it possible to show that homogentisic acid oxidase is also absent in alcaptonuric kidney but that the other enzymes involved in the oxidation of tyrosine to acetoacetic acid can be easily detected [90] Homogentisic acid oxidase could be demonstrated in nonalcaptonuric human kidney autopsy samples Thus the genetic defect in alcaptonuria is not limited to the synthesis of homogentisic acid oxidase in liver, it appears to affect the synthesis of the enzyme wherever it is normally present This is of theoretic interest, from the viewpoint of the genetic control and tissue specificity of enzymes [91 92] and it may be of some practical value in the detection of the carrier trait in relatives of alcaptonuric patients It might be possible to show a decreased amount of enzyme in carriers of the trait by a direct assay of the enzyme in a tissue more accessible than liver or kidney This would perhaps be a more accurate means to measure the enzyme than to determine it indirectly by the rate of metabolism of homogentisic acid in a tolerance test

The metabolic abnormality in alcaptonuria is present essentially from birth Garrod noted in 1901 [93] that staining of the diapers was scarcely

## INTERMITTENT ALCAPTONURIA

There are a few reports in the literature of intermittent alcaptonuria or instances in which it is reported that alcaptonuria has spontaneously disappeared—see Galdston et al [40] In view of the finding that alcaptonuria is associated with the lack of a pecific enzyme it is difficult to imagine how this hereditary condition would undergo intermittent exacerbations and remissions or a spontaneous cure Perhaps some of these cases of alcaptonuria are misdiagnosed and have some other reducing substance present in the urine Other cases may be examples in which some agent such as those described below which can induce experimental alcaptonuria in animals has altered the activity of the enzymes in this pathway with resultant homogentisic acid excretion Any further cases of alcaptonuria of this type should be carefully investigated with the specific methods now available to establish beyond any doubt that homogentisic acid is the reducing substance excreted in the urine In 1948 Fishberg reported [150] that a patient with autotoxic enterogenous cyanosis excreted up to ½ gm per day of benzoquinone acetic acid the quinone corresponding to homogentisic acid The amount excreted varied in inverse ratio to the ascorbic acid excretion She also found on the basis of nonspecific tests that patients with rheumatic fever and with scurvy excrete a similar quinone capable of producing methemoglobinemia These results have been questioned by Consden et al [151] who found no benzoquinone acetic acid excreted in scorbutic guinea pigs or in patients with rheumatic fever They suggest that bacterial activity in the urine leading to nitrite formation may have been responsible for the results of the qualitative tests employed by Fishberg The excretion of benzoquinone acetic acid in the case of enterogenous cyanosis cannot be explained in this way They suggest that the product might have been homogentisic acid which was oxidized to the quinone by nitrite arising from bacterial activity Others have found nitrite in the urine and blood of patients with enterogenous cyanosis [152]

## METABOLISM OF HOMOGENTISIC ACID

Under normal conditions no homogenti ic acid is present in the urine and none can be detected in plasma by the methods now available [70] Leaf and Neuberger [133] found that feeding as much as 5 gm homogentisic acid to normal adults produced no homogentisic aciduria However they found a transitory alcaptonuria following the intravenous injection of 0 3 or 1 0 gm homogentisic acid In these experiments the plasma concentration never rose above 1.5 mg per 100 ml plasma and it returned to normal values within 30 min

One might expect to find elevated pla ma levels of homogentisic acid in alcaptonuric patients in view of the large quantity of this acid ex

Homogentisic acid oxidase is inhibited by various quinones [106] by sulfhydryl binding agents [109] and by metal chelating agents such as $\alpha,\alpha'$-dipyridyl and o phenanthroline [119], which react with ferrous iron

Homogentisic acid oxidase activity can be measured manometrically, since the oxidation requires the uptake of two atoms of oxygen [97, 120] or it can be measured spectrophotometrically [97] by following the absorption of the product maleylacetoacetic acid at 330 mμ, providing the product is stable under the assay conditions The enzyme is found in the soluble fraction of liver and kidney [121] as are all the mammalian enzymes involved in the conversion of tyrosine to acetoacetic acid [122] Homogentisic acid oxidase activity is highest in liver, there is less activity in kidney, and no significant activity has been found in any of the other tissues so far examined [107 123] such as blood, salivary glands germinal epithelium and muscle This general distribution pattern has been found in rat rabbit, guinea pig, and pigeon [107] In man it is also highest in liver [16] and appreciable activity is also present in kidney [90] The liver of the toad, *Buffo marinus* has as high homogentisic acid oxidase activity as that found in mammalian liver [124] The presence of the enzyme in some microorganisms adapted to tyrosine has been previously mentioned [103 125]

The optimal pH for this enzyme is about 7 0 [104, 105] and it is specific for homogentisic acid Closely related compounds such as o-hydroxyphenylacetic acid p-hydroxyphenylacetic acid and gentisic acid are not oxidized [105] nor are homogentisic acid ethyl ester and homogentisic acid lactone The quinone formed by oxidizing homogentisic acid [127] does not appear to be an intermediate in the oxidation [105] and in fact this quinone is an inhibitor of the enzyme [105 126] The requirement for ferrous iron is apparently specific, since other bivalent metals such as $Co^{++}$ $Zn^{++}$ $Mg^{++}$ and $Mn^{++}$ cannot replace it [104 126]

A report in the German literature [128] that gentisic acid was less well metabolized in an alcaptonuric patient than in the normal person led Garrod to conclude that the enzyme system defective in alcaptonuria must catalyze the oxidation of some other 2,5-dihydroxyphenyl compounds as well as homogentisic acid [1] The specificity of the enzyme rules out this possibility and the reason for the results in the original study is not known It is possible that a larger percentage of gentisic acid is excreted as the free acid and less as conjugated derivatives in the alcaptonuric patient because of some inhibitory effect of homogentisic acid on the conjugation of gentisic acid and that for this reason less gentisic acid appeared to be metabolized However in one attempt to confirm this finding the excretion of free gentisic acid was found to be about the same in an alcaptonuric patient as in nonalcaptonuric persons [129]

of generalized ochronosis in the bones and connective tissues of cattle dogs and horses in which the tissues are described as being black as coal but again homogentisic acid has never been identified in the urine with certainty [20 136 137]

Experimental alcaptonuria has been produced in rats and mice by feeding large quantities of phenylalanine or tyrosine (see Table 12 2) It is also reported that vitamin C deficient guinea pigs fed extra phenylalanine [118 138] or tyrosine [118] excreted homogentisic acid as well as p-hydroxyphenylpyruvic acid and p-hydroxyphenyllactic acid Other workers however have found only the latter two compounds and no homogentisic acid in similar experiments with vitamin C–deficient guinea pigs [139] It should be noted that in some experiments of this type the claim has been made that homogentisic acid was excreted even though the analytical methods employed would not distinguish between p hydroxyphenylpyruvic acid and homogentisic acid [140]

Experimental alcaptonuria has been produced in rats and mice by feeding large quantities of phenylalanine and tyrosine (see Table 12 2)

TABLE 12 2 METHODS OF PRODUCING EXPERIMENTAL ALCAPTONURIA

| Agent | Species | Comment | Reference |
|---|---|---|---|
| Feeding L-phenylalanine | Rats | | [168 1~0] |
| | Mice | | [1 1] |
| | Guinea Pigs | Excreted HGA and PHPP on vitamin C–deficient diet defect corrected by vitamin C [118] | [138 118] |
| Feeding L-tyrosine | Rats | Believed by authors to be due to adaptive increase in tyrosine transamina e activity but decrease in homogentisic acid oxidase activity also found | [145] |
| | Guinea pigs | On vitamin C–deficient diet other workers find only PHPP PHPL no HGA [139] Defect corrected by ascorbic acid [118] or folic acid [1 1 3] | [11~ 118] |
| | Human being | Large doses over 1 day 50 gm 150 gm little or no HGA excreted | [141] |
| Diet deficient in sulfur amino acids | Rats | Effect reversed by cystine—not by ascorbic acid | [14 145] |
| α α Dipyridyl | Guinea pigs | Defect not altered by ascorbic acid | [104 1~4] |

NOTE HGA = homogentisic acid PHPP = p-hydroxyphenylpyruvic acid PHPL = p-hydroxyphenyllactic acid

creted per day Neuberger, Rimington and Wilson [27] found however that in a 7 year-old alcaptonuric girl the fasting plasma level was not more than about 3 mg per 100 ml plasma and that this level did not increase significantly following the oral administration of 3 gm L-phenyl alanine Nevertheless within 6 hr approximately 85 per cent of the given amino acid could be accounted for as homogentisic acid in the urine Neuberger et al made a very significant observation regarding the ex cretion of homogentisic acid during this investigation The plasma clearance data indicated that unless a large fraction of the urinary homogentisic acid were both synthe ized and excreted within the kidney glomerular filtration alone could not begin to account for the rate of homogentisic acid excretion In fact the clearance approached 400 to 500 ml per min about equal to the renal blood flow Even though it is most unusual for a normally occurring intermediate to be actively secreted by the kidney, this seems to be true of homogentisic acid This conclusion is in agreement with the earlier observation of Katsch and Metz [134] that intravenous homogentisic acid given to an alcaptonuric subject did not increase the plasma concentration significantly

More recent experiments in the author s laboratory [33] using an enzymatic assay to estimate plasma homogentisic acid have confirmed these conclusions Alcaptonuric and nonalcaptonuric individuals were found to excrete homogentisic acid rapidly after oral administration and the renal clearance data indicated active secretion by the kidney The possibility [27 135] that there might be an important difference in the renal handling of homogentisic acid between normal individuals and alcaptonuric patients can be dismissed

It appears that two factors serve to keep the plasma and presumably the tissue concentrations of homogentisic acid at a low level the great capacity to metabolize this acid in the liver and kidney and the rapid renal tubular secretion of homogentisic acid Even in the alcaptonuric patient the renal mechanism is capable of effectively lowering the plasma level when homogentisic acid is given This defense mechanism may be highly significant in view of the many years required for ochronosis to appear It is quite possible that in the alcaptonuric person the tissues are only occa ionally flooded with homogentisic acid and that this event has to be repeated many times over a period of years before tissue pigmentation occurs to a significant extent

## EXPERIMENTAL ALCAPTONURIA

Spontaneous alcaptonuria has not been found in any species except man Although there is a report by Lewis [136] of a rabbit with urine that darkened upon exposure to air and gave some of the qualitative tests for homogentisic acid the latter was never isolated or positively identified and the rabbit died without offspring There are also reports

by injecting rats intraperitoneally with L-tyrosine Under these conditions the activity of the enzymes later in the pathway was not changed significantly This adaptation is unusual, since it can also be produced by injecting hydrocortisone, by certain other amino acids and by propylene glycol [123] They also found that injections of L-tyrosine were not effective in adrenalectomized animals unless hydrocortisone was also given

In none of the methods for producing experimental alcaptonuria is the block in homogentisic acid oxidation complete In most only a small percentage of the homogentisic acid formed per day is excreted None of the methods is well suited for long term studies to produce experimental ochronosis and possibly arthritis secondary to alcaptonuria

## HEREDITARY ASPECTS

The first paper describing the inheritance of alcaptonuria was that of Garrod in 1902 [14] in which he presented evidence that this condition is congenital and familial and that it occurs more often in families in which there are consanguineous marriages He suggested that alcaptonuria might be transmitted as a single recessive Mendelian trait He believed that homogentisic acid arose in the normal course of tyrosine metabolism and that consanguineous marriages brought to light a recessive defect in this metabolic process In 1902 Bateson and Saunders [147] also suggested that the inheritance of a rare recessive factor might explain the incidence of alcaptonuria These studies on the mode of transmission of alcaptonuria were among the first on hereditary metabolic diseases

Although in the years immediately following examples were recorded in which direct transmission (i e parent and offspring affected) of alcaptonuria occurred Garrod believed that these were examples of a heterozygous individual mating with a homozygote As more family histories were described the general opinion of the recessive nature of the disease remained unchallenged In 1932 Hogben Worrall and Zieve [148] carefully summarized all the known cases of alcaptonuria reported up to that time Again the recessive character of the disease was confirmed in nearly all the families, and it was observed that at least half the affected individuals were the offspring of consanguineous matings Although there was an unequal sex distribution in their cases—100 males and 46 females—they did not consider this an indication that the condition was semilethal in females They noted that males were more often the probands in affected families and suggested that the higher incidence in males might be because of the more frequent examination of males They also noted that among infants there were slightly more females than males which was in agreement with this explanation Nevertheless it is frequently stated in medical texts that the incidence of alcaptonuria is twice as high

There is also a report of transitory homogentisic acid excretion after feeding a human volunteer large amounts of L-tyrosine [141]

Another type of experimental alcaptonuria has been induced in rats by a diet deficient in the sulfur-containing amino acids [142] This was not corrected by giving ascorbic acid but was reversed by giving cysteine [143] In this type of experimental alcaptonuria, proportionately less p hydroxyphenylpyruvic acid is excreted than in the type that responds to ascorbic acid [118]

In addition to the above methods the finding that homogentisic acid oxidase requires ferrous iron and can be inhibited by $\alpha\,\alpha'$-dipyridyl was used by Suda and Takeda [104] to induce experimental alcaptonuria in guinea pigs They injected $\alpha\,\alpha'$-dipyridyl and fed extra tyrosine The excretion of homogentisic acid in these animals was not corrected by administration of vitamin C

In another investigation, it was found that human volunteers on a vitamin C–deficient diet for several months with frank scorbutic symptoms did not excrete increased amounts of urinary phenols when given 20 gm tyrosine orally [144] This is further evidence that there is no direct requirement for ascorbic acid in homogentisic acid oxidation It can be concluded that the majority of instances of experimental alcaptonuria are either the result of direct inhibition of homogentisic acid oxidase or are due to an imbalance in the various enzyme reactions sufficient to cause an accumulation of homogentisic acid and its urinary excretion

Lin and Knox [145] have recently reported that in experimental alcaptonuria in rats induced by a diet supplemented with extra tyrosine no homogentisic acid was excreted for the first 3 or 4 days Following this initial lag period the degree of homogentisic acid excretion increased during the next several weeks They attribute these findings of the gradual increase in intensity of the alcaptonuria to an adaptive increase in tyrosine transaminase activity They believe that this type of alcaptonuria occurs because the relative rate of homogentisic acid formation overbalances the rate of homogentisic acid degradation Their data also show a significant decrease in liver homogentisic acid oxidase in the experimental group the decrease may be an important contribution to the resulting alcaptonuria

It is of interest to recall that the activity of the enzymes in the pathway following homogentisic acid oxidase was approximately normal in alcaptonuric liver (Table 12-1) Since the only known endogenous source of maleylacetoacetic acid is the oxidation of homogentisic acid, it appears that normal levels of the isomerase are produced in the absence of its substrate Thus it is a constitutive rather than an adaptive enzyme

Further studies should be mentioned in connection with the ' adaptive changes in the activity of liver tyrosine transaminase Lin and Knox [146] found that the level of this tran aminase could be increa veral fold

It would be most helpful if a diagnostic te t were available to detect the carrier state In view of the nature of the enzymatic defect in alcaptonuric individuals one might hope to find approximately one half of the normal amount of enzyme in the tissues of heterozygous individuals as is apparently the situation in phenylketonuria [153–155] and galactosemia [156 157] Measurements of the ability to metabolize homogentisic acid

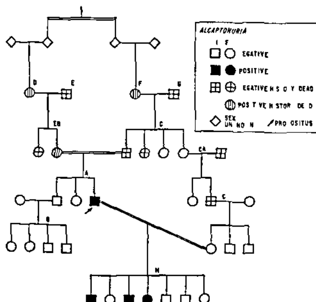


Fig 12 9 Alcaptonuria in a Lebane e family r ported by Khachadurian and Abu Feisal (By permi sion of 1 Khachad ian et al [150])

by an oral homogentisic acid tolerance test have so far shown no difference between relatives of alcaptonuric patients and normal controls [158] These results may be due to the tremendous capacity of the liver ( ee Table 12 1) to metabolize this acid Perhaps even if this reserve were reduced to one half it might not be detected by an oral tolerance test In fact assuming a liver weight of 1 500 gm and assuming that the liver homogenti ic acid oxida e is as efficient in vivo as under assay conditions in vitro it can be calculated that the normal adult liver can metabolize over 1 600 gm homogentisic acid per day

## TREATMENT

Attempts to treat alcaptonuria have been directed either toward cor recting the underlying metabolic defect or preventing or reversing the

in males as in females The paper by Hogben et al is obviously the source of this information

Among the families reviewed by Hogben et al there were some in which a dominant form of alcaptonuria had to be considered In a family studied by Pieter [149] the author felt compelled to conclude that a dominant type of alcaptonuria existed In the end this conclusion depended upon the predicted opportunity for marriage between homozygous and heterozygous individuals and the frequency of the heterozygote in *the general population or perhaps more exactly the incidence of the* heterozygote within a selected population The incidence of alcaptonuria in the general population can be only roughly estimated At least 200 cases have been described but this is a conservative number since new cases are generally not reported unless there are some other special features present It is reasonably certain however that alcaptonuria is less rare than was believed 30 years ago It is reported [9] that in a recent study in northern Ireland by A C Stephenson the incidence of alcaptonuria is from 3 to 5 per million individuals This would give a considerably higher incidence of heterozygous individuals in the general population than assumed by Hogben et al [148]

It is important to recall that in the families in which direct transmission of alcaptonuria has been found the number of consanguineous marriages is very high One of the best examples of this is the recently described kindred by Khachadurian and Abu Feisal [150] In this Lebanese family there was a total of seven alcaptonuric patients in four successive generations (Fig 12 9) Careful investigation however showed that the grandmothers of the propositus were first cousins and that at least two consanguineous marriages existed in this family This pedigree is particularly instructive because it illustrates how a recessive trait could appear to be a dominant one unless the entire family pedigree is known

At present most if not all cases appear to represent the inheritance of a single autosomal recessive gene This is supported by the biochemical finding that a single enzyme system is inactive in this condition and that only one clinical form of alcaptonuria is known The few cases in which it has been considered as possibly a dominant form have not shown any clinical differences from the majority of cases

The suggestion by Milch [151 152] that alcaptonuria is inherited as a dominant gene with incomplete penetrance seems unnecessarily complicated to explain the data at hand—see Knox [9] In fact while the possibility of a dominant type of alcaptonuria cannot be excluded it should be pointed out that no convincing evidence for it has yet been presented

The fact that a rare disease with recessive inheritance may be encountered more frequently in selected inbred populations complicates the estimation of the incidence of heterozygotes in the general population

It would be most helpful if a diagnostic test were available to detect the carrier state In view of the nature of the enzymatic defect in alcaptonuric individuals one might hope to find approximately one half of the normal amount of enzyme in the tissues of heterozygous individuals as is apparently the situation in phenylketonuria [*153–155*] and galactosemia [*156 157*] Measurements of the ability to metabolize homogentisic acid

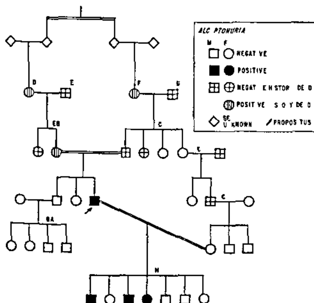



Fig 12 9 Alcaptonuria in a Lebanese family reported by Khachadurian and Abu Feisal (*By permission of A Khachadurian et al* [*150*])

by an oral homogentisic acid tolerance test have so far shown no difference between relatives of alcaptonuric patients and normal controls [*158*] These results may be due to the tremendous capacity of the liver (see Table 12 1) to metabolize this acid Perhaps even if this reserve were reduced to one-half it might not be detected by an oral tolerance test In fact assuming a liver weight of 1,500 gm and assuming that the liver homogentisic acid oxidase is as efficient in vivo as under assay conditions in vitro it can be calculated that the normal adult liver can metabolize over 1 600 gm homogentisic acid per day

## TREATMENT

Attempts to treat alcaptonuria have been directed either toward correcting the underlying metabolic defect or preventing or reversing the

pigmentation and arthritic changes Galdston Steele and Dobriner [40] administered several vitamins brewers yeast tyrosinase insulin and adrenocortical extract without altering the amount of homogentisic acid excreted by an alcaptonuric patient Several groups have studied the effectiveness of vitamin C [26, 27, 40, 159, 160] Although it corrects the alcaptonuria induced in guinea pigs by feeding large amounts of tyrosine [117] it does not change the hereditary type of alcaptonuria

Other agents, such as vitamin $B_{12}$ [161], cortisone [19 162, 163] and phenylbutazone [164], are without influence on the metabolic defect A confusing report by Cope and Kassander [56] that cortisone corrected the metabolic error is difficult to interpret since the authors claim this was a case of ochronosis without homogentisic acid in the urine

Now that it is certain that the basic defect is the lack of a specific enzyme replacement of the missing enzyme is theoretically a therapeutic measure to consider, but this is not practical at present Even if pure homogentisic acid oxidase were available in large quantities its administration might very well cause an antibody response capable of inactivating the enzyme It is possible that a synthetic chemical compound might be found which could replace the missing enzyme, but no such catalyst is now available

In the past it has been suggested that dietary phenylalanine and tyrosine be reduced to decrease the output of homogentisic acid A severe restriction of the intake of these amino acids is not practical except for brief periods and might be dangerous to the patient if continued over a long time

Since from a practical standpoint the importance of the metabolic defect is mainly that it leads to pigmentation and arthritic changes therapeutic measures could be aimed primarily at preventing or correcting these complications of the disease It is possible as Sealock Galdston and Steele have pointed out that large amounts of ascorbic acid might prevent the deposition of ochronotic pigment [26] even though this does not alter the metabolic defect

## SUMMARY

1 Alcaptonuria is a rare hereditary metabolic disease in which homogentisic acid an intermediary product in the metabolism of phenylalanine and tyrosine cannot be further metabolized The metabolic defect causes a characteristic triad of homogentisic aciduria, ochronosis and arthritis

2 The cause of the disease is a constitutional lack of the enzyme homogentisic acid oxidase This enzyme normally exists primarily in the liver and kidney It requires oxygen ferrous ion and sulfhydryl groups for opening the ring of homogentisic acid

3 The condition is inherited as an autosomal recessive disease No method for the detection of heterozygotes has been devised

4 The relationships between the metabolic defect and the complica tions ochronosis and arthritis remain a challenging research problem of the future Even though the lack of homogentisic acid oxidase is no doubt the ultimate cau e of the e complications the mechanisms which bring them about are unknown

## BIBLIOGRAPHY

1 Garrod A E The Croonian lectures on inborn errors of metabolism Lecture II Alkaptonuria Lancet 2 73 1908
2 Boedeker C Ueber das Alcapton ein neuer Beitrag zur Frage welche Stoffe des Harns konnen Kupferreduction bewirken? Ztschr rat Med 7 130 1859
3 Boedeker C Das Alkapton ein Beitrag zur Frage welche Stoffe des Harns konnen aus einer alkalischen Kupferoxydlosung Kupferoxydul reduciren? Ann Chem u Pharm 117 98 1861
4 Ebstein W and Muller J Brenzkatechin in dem Urin eines Kindes Arch path Anat 62 554 1875
5 Furbringer P V *Nachtrag uber Alkaptonurie Berl klin Wchnschr* 12 390 1875
6 Smith W G On the occurrence of protocatechuic acid in urine Dublin J M Sc 73 465 1882
7 Marshall J A preliminary noti e of a crystalline acid in urine possessing more powerful reducing properties than glucose M News (Philadelphia) 50 35 1887
8 Kirk R On a new acid found in human urine which darkens w th alkalies J Anat & Physiol London 23 69 1889
9 Knox W E Sir Archibald Garrod s inborn errors of metabolism II Alkaptonuria Am J Human Genet 10 95 1958
10 Wolkow M and Baumann E Über das Wesen der Alkaptonurie Ztschr physiol Chem 15 228 1891
11 Garrod A E *Inborn Errors of Metabolism* Frowde Hodder and Stoughton London 1909
12 Neubauer O Über den Abbau der Aminosauren im gesunden und kranken Organi mus Deutsches Arch klin Med 95 211 1909
13 Neubauer O Intermediarer Eiweisstoffwechsel Handb norm path Physiol 5 671 1928
14 Garrod A E The incidence of alkaptonuria a study in chemical individuality Lancet 2 1616 1902
15 Beadle G W and Tatum F L Genetic control of biochem cal r actions in neuro pora Proc Nat Acad Sc U S 27 499 1941
16 La Du B N Zannoni V G Laster L and Seegmiller J E The nature of the d f ct in tyro ine metaboli m in alcaptonuria J Biol Chem 230 251 1958
17 Cooper P A Alkaptonuria with o hrono is Proc Roy Soc Med 44 917 1951
18 Minno A M and Rogr J A Ochrono is repo t of a case Ann Int Med 46 1 9 1957
19 Yules J H Ochronotic arthritis Report of a case Bull New England M Cente 16 168 1954
20 Martin W J Underdahl L O and Mathieson D R Alkaptonuria report of 3 cases Proc Staff Meet Mayo Clin 27 193 1952
21 Crissey R E and Day A J Ochrono is a ase report J Bone & Joint Surg 32A 688 1950
22 Smith H P and Smith H I Jr Ochronosis report of two cases Ann Int Med 42 171 1955
23 Hammond G and Powers H W Alkaptonuric arthritis report of a case Lahey Clin Bull 11 18 1958

24 Pomeranz M M Friedman L J and Tunick I S Roentgen findings in alcaptonuric ochronosis Radiology 37 295 1941
25 Rose G K Ochronosis Brit J Surg 44 481 1957
26 Sealock R R Galdston M and Steele J M Administration of ascorbic acid to an alkaptonuric patient Proc Soc Exper Biol & Med 44 580 1940
27 Neuberger A Rimington C and Wilson J M C Studies on alcaptonuria II Investigations on a case of human alcaptonuria Biochem J 41 438 1947
28 Briggs A P A colorimetric method for the determination of homogentisic acid in urine J Biol Chem 51 453 1922
29 Fishberg F H The instantaneous diagnosis of alkaptonuria on a single drop of urine J A M A 119 882 1942
30 Neuberger A Studies on alcaptonuria I The estimation of homogentisic acid Biochem J 41 431 1947
31 Medes G Modification of Garrod s method for preparation of homogentisic acid from urine Proc Soc Exper Biol & Med 30 751 1933
32 Knox W E and LeMay Knox M The oxidation in liver of L-tyrosine to acetoacetate through p-hydroxyphenylpyruvate and homogentisic acid Biochem J 49 686 1951
33 Seegmiller J E and La Du B N Unpublished
34 La Du B N and O Brien W M Unpublished
35 Virchow R Ein Fall von allgemeiner Ochronose der Knorpel und knorpelähnlichen Theile Arch path Anat 37 212 1866
36 Albrecht H Ueber Ochronose Ztschr Heilk 23 366 1902
37 Osler W Ochronosis the pigmentation of cartilages sclerotics and skin in alkaptonuria Lancet 1 10 1904
38 Bunim J J McGuire J S Jr Hilbish T F Laster L La Du B N Jr and Seegmiller J E Alcaptonuria clinical staff conference at the National Institutes of Health Ann Int Med 47 1210 1957
39 Smith J W Ochronosis of the sclera and cornea complicating alkaptonuria review of the literature and report of four cases J A M A 120 1282 1942
40 Galdston M Steele J M and Dobriner K Alcaptonuria and ochronosis with a report of three patients and metabolic studies in two Am J Med 13 432 1952
41 Lichtenstein L and Kaplan L Hereditary ochronosis Pathological changes observed in two necropsied cases Am J Pathol 30 99 1954
42 Cooper J A and Moran T J Studies on ochronosis A M A Arch Pathol 64 46 1957
43 Fitzpatrick T B and Lerner A B Biochemical basis of human melanin pigmentation A M A Arch Dermatol 69 133 1954
44 Lillie R D A Nile blue staining technic for the differentiation of melanin and lipofuscins Stain Technol 31 151 1956
45 Morner K A H Zur Kenntnis von den Farbstoffen der melanotischen Geschwulste Ztschr physiol Chem 11 66 1887
46 Milch R A Titus E D and Loo T L Atmospheric oxidation of homogentisic acid spectrophotometric studies Science 126 209 1957
47 Milch R A and Titus E D Studies of alcaptonuria absorption spectra of homogentisic acid-chondroitin sulfate solutions Arthritis & Rheumat 1 566 1958
48 Sugar H S and Waddell W W Ochronosis like pigmentation associated with the use of atabrine Illinois M J 89 234 1946
49 Pick L Ueber die Ochronose Berl klin Wchnschr 43 591 1906
50 Reid E Osler W and Garrod A E On ochronosis Quart J Med 1 199 1908
51 Pope F M A case of ochronosis With a note on the relationship of alkaptonuria to ochronosis by A E Garrod Lancet 1 24 1906
52 Beddard A P Ochronosis associated with carboluria Quart J Med 3 329 1910
53 Beddard A P and Plumtre C M A further note on ochronosis associated with carboluria Quart J Med 5 505 1912
54 Brogren N Case of exogenetic ochronosis from carbolic acid compresses Acta dermat venereol 32 258 195_

55 Oppenheimer B S and Kline B S Ochronosis with a study of an additional case Arch Int Med 29 73? 19 ?
56 Cope C B and Kassander P Cortisone in ochronotic arthritis J A M A 150 997 195?
57 Janney N W A study of ochronosis Am J M Sc 156 59 1918
58 Skinsnes O K Generalized ochronosis report of an instance in which it was misdiagnosed as melanosarcoma with resultant enucleation of an eye Arch Path 45 55? 1948
59 Furbringer P Beobachtungen über einen Fall von Alkaptonurie Berl klin Wchnschr 12 330 18?5
60 Moraczewski W von Ein Fall von Alkaptonurie Centr inn Med XVII 177 1896
61 Harrold A J Alkaptonuric arthritis J Bone & Joint Surg 38B 532 1956
6? Hench P S Rheumatism and arthritis review of American and English literature of recent years 9th Rheumatism Review Ann Int Med 28 310 1948
63 Sutro C J and Anderson M E Alkaptonuric arthritis cause for free intra articular bodies Surgery 22 1?0 1947
64 Greiling H Beitrag zur Entstehung der Ochronose bei Alkaptonurie Klin Wchnschr 35 889 195?
65 Coodley E L and Greco A J Clinical aspects of ochronosis with report of a case Am J Med 8 816 1950
66 Eisenberg H Alkaptonuria ochronosis arthritis and ruptured intervertebral disk A M A Arch Int Med 86 ?9 1950
67 *Young H H Calculi of the prostate associated with ochronosis and alkaptonuria* J Urol 51 48 1944
68 Rosenbaum H and Reveno W S Polycythemia and alkaptonuria Harper Hosp Bull 10 36 195?
69 Dalgliesh C E Metabolism of the aromatic amino acids Advances Protein Chem 10 31 1955
?0 Knox W E The metabolism of phenylalanine and tyrosine in *A Symposium on Amino Acid Metabolism* edited by W D McElroy and H B Glass Johns Hopkins Press Baltimore 1955
71 Lerner A B Metabolism of phenylalanine and tyrosine Advances Enzymol 14 ?3 1953
7? Meister A *Biochemistry of the Amino Acids* Academic Press Inc New York 1957
73 Schepartz B and Gurin S The intermediary metabolism of phenylalanine labeled with radioactive carbon J Biol Chem 180 663 1949
74 Weinhouse S and Millington R H Ketone body formation from tyrosine J Biol Chem 175 995 1948
?5 Weinhouse S and Millington R H Ketone body formation from tyrosine J Biol Chem 181 645 1949
?6 Dische R and Rittenberg D The metabolism of phenylalanine-4-C J Biol Chem 211 199 1954
?7 Lerner A B On the metabolism of phenylalanine and tyrosine J Biol Chem 181 281 1949
?8 Bamberger F Über das Verhalten paraalkylierter Phenole gegen das Caroscles Reagens Ber deut chem Ges 36 ? 8 1903
?9 Dakin H D The chemical nature of alkaptonuria J Biol Chem 9 151 1911
80 Dakin H D Experiments relating to the mode of decomposition of tyrosine and of related substances in the animal body J Biol Chem 8 11 1910
81 Wakeman A J and Dakin H D The catabolism of phenylalanine tyrosine and of their derivatives J Biol Chem 9 139 1911
8? La Du B N and Zannoni V G The tyrosine oxidation system of liver II Oxidation of p-hydroxyphenylpyruvic acid to homogentisic acid J Biol Chem 217 ? ? 1955
83 Pirrung J Gottesman L and Crandall D I The metabolism of p-methoxyphenylalanine and p-methoxyphenylpyruvate J Biol Chem 229 199 1957

84 Recknagel R O and Potter V R Mechanism of the ketogenic effect of ammonium chloride J Biol Chem 191 263 1951
85 Edwards S W Hsia D Y Y and Knox W E The first oxidative enzyme of tyrosine metabolism p-hydroxyphenylpyruvate oxidase Fed Proc 14 206 1955
86 Neuberger A Synthesis and resolution of 2,5-dihydroxyphenylalanine Biochem J 43 599 1948
87 Gross O Über den Einfluss des Blutserums des Normalen und des Alkaptonurikers auf Homogentisinsäure Biochem Ztschr 61 165 1914
88 Katsch G, and Stern G Zur Theorie der alkaptonurischen Stoffwechselstörung Deutsches Arch klin Med 151 329 1926
89 Lanyar F and Lieb H Die quantitative Bestimmung der Homogentisinsäure im Blutserum und in der Milch des Alkaptonurikers Ztschr physiol Chem 203 135 1931
90 La Du, B et al Unpublished observations
91 Henion W F and Sutherland E W Immunological differences of phosphorylase J Biol Chem 224 477, 1957
92 Schlamowitz M Immunochemical studies on alkaline phosphatase in Symposium on Enzymes in Blood Ann New York Acad Sc 75 373 1958
93 Garrod A E About alkaptonuria Lancet 2 1484 1901
94 Kretchmer N Levine, S Z McNamara H and Barnett H L Certain aspects of tyrosine metabolism in the young I The development of the tyrosine oxidizing system in human liver J Clin Invest 35 236 1956
95 Braid F and Hickmans L M Metabolic study of an alkaptonuric infant Arch Dis Childhood 4 389 1929
96 Mittelbach F Ein Beitrag zur Kenntnis der Alkaptonurie Deutsches Arch klin Med 71, 50 1901
97 Knox W E and Edwards S W Homogentisate oxidase of liver J Biol Chem 216 479 1955
98 Knox W E and Edwards S W The properties of maleylacetoacetate the initial product of homogentisate oxidation in liver J Biol Chem 216, 489 1955
99 Edwards S W and Knox W E Homogentisate metabolism the isomerization of maleylacetoacetate by an enzyme which requires glutathione J Biol Chem 220 79 1956
100 Ravdin R G and Crandall D I The enzymatic conversion of homogentisic acid to 4 fumarylacetoacetic acid J Biol Chem 189 137 1951
100a Meister A and Greenstein J P Enzymatic hydrolysis of 2 4-diketo acids J Biol Chem 175 573 1948
101 Witter R F and Stotz E The metabolism in vitro of triacetic acid and related diketones J Biol Chem 176 501 1948
102 Connors W M and Stotz E The purification and properties of a triacetic acid hydrolyzing enzyme J Biol Chem 178 881 1949
103 Suda M and Takeda Y Metabolism of tyrosine I Application of successive adaptation of bacteria for the analysis of the enzymatic breakdown of tyrosine J Biochem (Japan) 37 3 5 1950
104 Suda M and Takeda Y Metabolism of tyrosine II Homogentisicase J Biochem (Japan) 37 381 1950
105 Crandall D I Homogentisic acid oxidase II Properties of the crude enzyme in rat liver J Biol Chem 212 565 1955
106 Schepartz B Inhibition and activation of the oxidation of homogentisic acid J Biol Chem 205 185 1953
107 Crandall D I and Halikis D N Homogentisic acid oxidase I Distribution in animal tissues and relation to tyrosine metabolism in rat kidney J Biol Chem 208 629 1954
108 Crandall D I Yasunobu K Krueger R C and Mason H S Oxygen transfer by homogentisate oxidase Fed Proc 17 207 1958
109 Crandall D I in Symposium on Amino Acid Metabolism edited by W D McElroy and H B Glass p 867 Johns Hopkins Press Baltimore 1955

110 Suda M Hashimoto K Matsuoka H and Kamahora T Further studies on pyrocatecase J Biochem (Japan) 38 289 1951
111 Stanier R Y and Ingraham J L Protocatechuic acid oxidase J Biol Chem 210 799 1954
112 Long C L Hill H N Weinstock I M and Henderson L M Studies of the enzymatic transformation of 3-hydroxyanthranilate to quinolinate J Biol Chem 211 405 1954
113 MacDonald D L Stanier R Y and Ingraham J L The enzymatic formation of β-carboxymuconic acid J Biol Chem 210 809 1954
114 Dagley S and Patel M D Microbial oxidation of p-cresol and protocatechuic acid Biochem J 60 XXXV 1955
115 Mason H S Mechanisms of oxygen metabolism Advances Enzymol 19 79 1957
116 Suda M Homogentisic acid oxidizing enzyme M J Osaka Univ 8 Suppl 57 1958
117 Sealock R R and Silberstein H E The control of experimental alcaptonuria by means of vitamin C Science 90 517 1939
118 Sealock R R and Silberstein H E The excretion of homogentisic acid and other tyrosine metabolites by the vitamin C-deficient guinea pig J Biol Chem 135 251 1940
119 Schepartz B Intermediate steps in tyrosine metabolism Fed Proc 12 265 1953
120 Edwards S W and Knox W E in *Methods in Enzymology* edited by S P Colowick and N O Kaplan vol 2 p 292 Academic Press Inc New York 1955
121 Crandall D I L-tyrosine oxidation in rat kidney Fed Proc 13 195 1954
122 Knox W E in *Methods in Enzymology* edited by S P Colowick and N O Kaplan vol 2 p 287 Academic Press Inc New York 1955
123 Lin E C C and Knox W E Specificity of the adaptive response of tyrosine-α ketoglutarate transaminase in the rat J Biol Chem 233 1180 1958
124 La Du B N and Zannoni V G Unpublished observations
125 Jones J D Smith B S and Evans W C Homogentisic acid an intermediate in the metabolism of tyrosine by the aromatic ring-splitting microorganisms Biochem J 51 XI 195
126 Crandall D I Properties and distribution of homogentisic acid oxidase Fed Proc 12 19 1953
127 Morner C T Weitere Beitrage zur Chemie der Homogentisinsäure Ztschr physiol Chem 78 306 1912
128 Neubauer O and Falta W Über das Schicksal einiger aromatischer Sauren bei der Alkaptonurie Ztschr physiol Chem 42 81 1904
129 La Du B et al Unpublished observations
130 Fishberg E H Excretion of benzoquinoneacetic acid in hypovitaminosis C J Biol Chem 172 155 1948
131 Consden R Forbes H A W Glynn L E and Stanier W M Observations on the oxidation of homogentisic acid in urine Biochem J 50 274 1951
132 Evans A S Enzer N Eder H A and Finch C A Hemolytic anemia with paroxysmal methemoglobinemia and sulfhemoglobinemia A M A Arch Int Med 86 22 1950
133 Leaf G and Neuberger A The preparation of homogentisic acid and of 2,5-dihydroxyphenylethylamine Biochem J 43 606 1948
134 Katsch G and Metz L Der Nachweis der Homogentisinsäure im Serum des Alkaptonurikers Deutsches Arch klin Med 157 143 1927
135 Dent C E ed Symposium on inborn errors of metabolism Am J Med 22 671 1957
136 Lewis J H Alcaptonuria in a rabbit J Biol Chem 70 651 1926
137 Poulsen V Über Ochronose bei Menschen und Tieren Beitr path Anat 48 346 1910
138 Sealock R R Perkinson J D Jr and Basinski D H Further analysis of

the role of ascorbic acid in phenylalanine and tyrosine metabolism J Biol Chem 140 153 1941
139 Painter H A and Zilva S S The influence of L-ascorbic acid on the disappearance of the phenolic group of L-tyrosine in the presence of guinea pig liver suspensions Biochem J 46 542 1950
140 Malakar M C and Banerjee S N Effect of glycine or choline chloride on the excretion of homogentisic acid by the tyrosine fed and scorbutic guinea pigs Ann Biochem & Exper Med 15 63 1955
141 Abderhalden E Bildung von Homogentisinsaure nach Aufnahme grosser Mengen von l Tyrosin per os Ztschr physiol Chem 77 454 1912
142 Glynn L E Himsworth H P and Neuberger A Pathological states due to deficiency of the sulfur containing amino-acids Brit J Exper Path 26 326 1945
143 Neuberger A and Webster T A Studies on alcaptonuria 3 Experimental alcaptonuria in rats Biochem J 41 449 1947
144 Bartley W Krebs H A and O'Brien J R P Vitamin C requirement of human adults Medical Research Council Special Report Series No 280 p 24 Her Majesty's Stationery Office London 1953
145 Lin E C C and Knox W E Role of enzymatic adaptation in production of experimental alkaptonuria Proc Soc Exper Biol & Med 96 501 1957
146 Lin E C C and Knox W E Adaptation of the rat liver tyrosine-α-ketoglutarate transaminase Biochim et biophys acta 26 85 1957
147 Bateson W and Saunders E R *Report of the Evolution Committee of the Royal Society (London)* No 1 p 133 1902 Cited by Garrod (ref 14)
148 Hogben L Worrall R L and Zieve I The genetic basis of alkaptonuria Proc Roy Soc Edinburgh 52 264 1932
149 Pieter H Une famille d'alcaptonuriques Presse méd 33 1310 1925
150 Khachadurian A and Abu Feisal K Alkaptonuria Report of a family with seven cases appearing in four successive generations with metabolic studies in one patient J Chron Dis 7 455 1958
151 Milch R A Direct inheritance of alcaptonuria Metabolism 4 513 1955
152 Milch R A Inheritance of alcaptonuria Bull Hosp Joint Dis 18 103 1957
153 Hsia D Y Y Driscoll K W Troll W and Knox W E Detection by phenylalanine tolerance tests of heterozygous carriers of phenylketonuria Nature London 178 1239 1956
154 Hsia D Y Y and Paine R S Phenylketonuria detection of the heterozygous carrier J Ment Deficiency Res 1 53 1957
155 Knox W E and Messinger E C The detection in the heterozygote of the metabolic effect of the recessive gene for phenylketonuria Am J Human Genet 10 53 1958
156 Holzel A and Komrower G M A study of the genetics of galactosaemia Arch Dis Childhood 30 155 1955
157 Kirkman H N and Bynum E Enzymic evidence of a galactosemic trait in parents of galactosemic children Ann Human Genet 23 117 1959
158 La Du B et al Unpublished observations
159 Mosonyi L A propos de l'alcaptonurie et de son traitement Presse méd 47 708 1939
160 Díaz C J Mendoza H C and Rodríguez J S Alkapton Aceton und Kohlehydratmangel Klin Wchnschr 18 965 1939
161 Flaschentrager B Halawani A and Nabeh I Alkaptonurie und Vitamin $B_2$ Klin Wchnschr 32 131 1954
162 Black R L Use of cortisone in alkaptonuria J A M A 155 968 1954
163 Suzman M M The clinical application of corticotropin and cortisone therapy a report of 247 cases S African M J 27 135 1953
164 Biggs T G Jr and Cannon E Jr Ochronosis report of a case J Louisiana State M Soc 105 395 1953
165 *The Oxford English Dictionary Supplement* p 15 Oxford New York 1933
166 Sakamoto Y Nakamura K Inamori K Ikeda S and Ichihara K On the formation of gentisic acid II J Biochem (Japan) 44 849 1957

167 Ichihara K Ikeda S and Sakamoto Y On the formation of gentisic acid from homogentisic acid by the liver extract J Biochem (Japan) **43** 129 1956
168 Papageorge E and Lewis H B Experimental alcaptonuria in the white rat J Biol Chem **123** 211 1938
169 Butts J Dunn M S and Hallman L F Studies in amino acid metabolism IV Metabolism of dl phenylalanine and dl tyrosine in the normal rat J Biol Chem **123** 711 1938
170 Lanyar F Über experimentelle Alkaptonurie bei der Weissen Ratte Ztschr physiol Chem **278** 155 1943
171 Lanyar F Über experimentelle Alkaptonurie bei der Weissen Maus Ztschr physiol Chem **275** 225 1942
172 Woodruff C W Cherrington M E Stockell A K and Darby W J The effect of pteroylglutamic acid and related compounds upon tyrosine metabolism in the scorbutic guinea pig J Biol Chem **178** 861 1949
173 Woodruff C W and Darby W J An *in vivo* effect of pteroylglutamic acid upon tyrosine metabolism in the scorbutic guinea pig J Biol Chem **172** 851 1948
174 Suda M Takeda Y Sujishi K and Tanaka T Metabolism of tyrosine III Relation between homogentisicase ferrous ion and L-ascorbic acid in experimental alcaptonuria of guinea pig J Biochem **38** 297 1951

# Chapter 13

# Albinism*

*Thomas B Fitzpatrick*

Albinism is an inherited disorder of melanin metabolism characterized by a decrease or absence of melanin in the skin hair and eyes Because *the clinical features are so striking the condition was recognized early*, and accurate descriptions of albinos are found in the writings of Pliny and Ptolemy The term *albino* is derived from the Latin adjective *albus* white, and was first used by Balthazar Tellez for certain white Negroes seen by him and other traders on the African coast An historical review of albinism can be found in the monographs by Pearson, Nettleship and Usher [1] (1911–1913) and a more recent well-documented summary by Froggatt [2] The essential clinical features of albinism are well described in the following paragraphs written by Lionel Wafer [3] in 1699

> There is one complexion so singular that I never saw nor heard of any like them in any part of the world they are white tis a milk white lighter than the colour of any Europeans and much like that of a white horse their seeing so clear as they do in a moon shiny night we used to call them moon-eyed For they see not very well in the sun poring in the clearest day (their eyes being but weak and running with water if the sun shine towards them ) so that in the day time they care not to go abroad when the moon shiny nights come they are all life and activity running abroad and into the woods (skipping about like wild bucks and running as fast by moon light ) even in the gloom and shade of the woods as the other Indians by day being as nimble as they tho not so strong and lusty (neither is the child of a man and woman of these white Indians white like the parents but copper coloured as their parents were) and they were but short lived

Outstanding characteristics are the milk white color and marked photophobia with eyes being but weak and running with water if the sun shine towards them The recessive inheritance is suggested by

This chapter was written while the author was in Oxford England as a Fellow of the Commonwealth Fund

neither is the child of a man and woman of these white Indians white like the parents but copper coloured The marked susceptibility of albinos to skin cancer with meta tases and death is expressed in the phrase ' they were but short liv d The striking contrast to the normal dark skin in Indians and Negroes set them apart and many myths were conjured up about these strange 'moon ey d people, "skipping about like wild bucks, and running as fast by moon light ' Even today in Panama in the mythology of primitive Indian tribes there are several prominent albino heroes and albinos are believed to have a special aura

Garrod [4] with judicious prescience included albini m among his inborn errors of metabolism His speculations on the nature of the metabolic defect in albinism and the mechanism of melanin formation are truly remarkable

> Three possible explanations of the phenomenon of albinism suggest them selves We might suppose that the cells which usually contain pigment fail to take up melanins formed elsewhere or that the albino has an unusual power of destroying these pigments or again that he fails to form them
>
> It is very unlikely that the melanin is conveyed to the pigmented cells and there deposited for all the evidence indicates that the pigment is formed *in situ* probably by the action of intracellular enzymes
> Only certain specialized cells appear to have the power of forming melanin
>
> Taking all the known facts into consideration the theory that what the albino lacks is the power of forming melanin which is normally possessed by certain specialized cells is that which has most in its favour and is probably the true one If so an intracellular enzyme is probably wanting in subjects of this anomaly an explanation which brings albinism into line with some other inborn metabolic errors of which a similar explanation is at least a possible one [4]

This chapter will be largely devoted to a proof of Garrod s theory ba ed on present knowledge of melanin formation For purposes of orientation before discussing the biochemical lesion of albinism the characteristic clinical features of the various types of albinism will be described

## DEFINITION AND CLASSIFICATION

Albinism is an inherited metabolic defect of a pigment cell or *melanocyte* resulting in a failure of this cell to form melanin

The melanocyte is a specialized distinctive dendritic shaped cell with two or more cytoplasmic processes surrounding a perikaryon Melanocytes are derived from the neural crest[1] and are normally located

[1] The retinal pigment epithelial cells are derived from the outer layer of the optic cup The pigment cells have a morphology different from that of melanocytes elsewhere Electron micrographs of the melanin granules of the retinal pigment epithelium show a morphology similar to that of melanin granules of the hair bulb The retinal pigment epithelium conta ns tyro na e but only during the first few weeks of development [5]

in man in certain characteristic regions *skin* (at the epidermal-dermal junction) *hair bulb nervous system* (in the pia-arachnoid) and *eye* (uveal tract and retinal pigment epithelium) The melanocytes constitute an embryologic, morphologic and biochemical unit referred to as the *melanocyte system* (Fig 13 1) With the curious exception of the melanocytes of the hair bulb and retinal pigment epithelium, all melanocytes have the propensity to form malignant melanomas

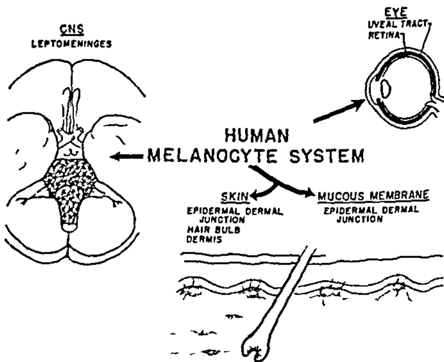



Fig 13 1 The human melanocyte system

The metabolic defect of the melanocyte in albinism may involve the entire melanocyte system or only one locus of melanocytes For example albinism may be limited to the retinal pigment epithelium Classification therefore should refer to the *extent* of the involvement of the melanocyte system Numerous terms have been used including complete and incomplete perfect and imperfect and partial Table 13 1 gives the terminology recommended here and some of the terms which have been used Since the adjectives describe the extent of the metabolic defect throughout the melanocyte system the terms *universal generalized* and *localized* are more acceptable than complete imperfect partial etc [1 6, 7]

In order to classify albinism as *universal* histologic examination of the entire melanocyte system must be made to prove the absence of melanin

TABLE 13 1 CLASSIFICATION OF ALBINISM

| *Recommended terminology* | *Present terminology* | *Description* | *Heredity* |
|---|---|---|---|
| Universal albinism | Complete albinism<br>Perfect albinism | Absence of melanin in the hair bulb skin uveal tract and retinal pigment epithelium | Autosomal recessive |
| Generalized albinism | Incomplete albinism<br>Imperfect albinism | Absence of melanin in the skin hair and retinal pigment epithelium but presence of melanin in the iris and occasionally the skin | Autosomal recessive<br>Irregular dominant |
| Localized albinism | | | |
| Of the skin | Partial albinism<br>Piebaldism | Absence of melanin in scattered isolated areas of skin and hair | Autosomal dominant |
| | White forelock | Absence of melanin in the hair of the frontal region | |
| Of the eye | Ocular albinism | Absence of melanin in the retinal pigment epithelium | Sex linked recessive |

Affecting the whole of something specified *The Shorter Oxford English Dictionary* Oxford University Press 1956

A clinical diagnosis of universal albinism cannot be made It occurs in animals but none of the autopsy studies on human albinos have proved universal absence of melanin in man

A diagnosis of *generalized* albinism requires that there be ocular involvement (nystagmus photophobia decreased visual acuity and translucent irises)

## CHARACTERISTICS IN MAN

### INCIDENCE

Albinism is widespread in the animal kingdom It is found in fish birds and mammals [8 9 10]

Generalized albinism in man is not uncommon The incidence has been reported to be from 1 5 000 to 1 25 000 The highest incidence (5 1 000) was found in the San Blas Indians of Panama [11] There are approximately 700 pedigrees of generalized albinism in the literature It has been reported in all races

The incidence of localized albinism of the eye skin or hair is not known Approximately twenty four families with localized cutaneous and pilary albinism (*partial albinism*) had been reported as of 1954 [12]

Both generalized and localized albinism are usually recognized by the dermatologist who is consulted because of solar intolerance or by the ophthalmologist who is consulted because of ocular problems Since many

generalized albinos do not seek the attention of these specialists, the incidence may be considerably higher than existing figures indicate

CLINICAL FEATURES

*Universal and generalized albinism*

The generalized albino exhibits milk white skin color and whitish yellow hair, and has a characteristic facies because of photophobia and habitual squinting (Fig 13 2)



Fig 13 2 Generalized albinism in a Negro

Skin *Color* In all races (Caucasian Asiatic, Indian and Negroid), the skin color of the albino is milk white The reflectance of Caucasian albino skin as measured by a reflectance meter [*13*] is no greater than that of un tanned normal skin In the African Negro albino, Barnicot [*14*] compared the color visually to that of a lightly tanned European, while in the case of African albino infants the skin is "very white"

In the Negroid race particularly there is a peculiar inherited pigmentary change known as "xanthism" The hair has a strong *red* color and the skin is a bright *copper red* The iris color is diluted Barnicot [*15*] believes that the condition is distinct from generalized albinism but Pearson et al [*1*] cite a pedigree in which both albinism and xanthism appear

*Response to Solar Exposure* In both the Caucasian and Negro albino the skin of the exposed areas may be markedly erythematous, and in the African albino it may be wrinkled and folded particularly on the back of the neck and arms [*14*] This change in surface contour is absent in African albino infants and thus may result from chronic solar exposure In adult albinos exposed to prolonged intense solar radiation precancerous keratoses and squamous and basal carcinomas are almost invariably found [*16*] Carcinoma of the exposed surfaces is extremely rare in pigmented peoples (Asiatic Indian and Negro) The demographic data of MacDonald [*17*] and others [*18*] strongly implicate solar ultraviolet radiation as a carcinogenic agent in the Caucasian The susceptibility of the human albino skin and albino mouse to solar and artificial ultraviolet radiant energy and the relative immunity of pigmented peoples and

pigmented mice [19] indicate the important role of melanin Thomson [20] has demonstrated the remarkable opacity of isolated Negro corneum to solar radiation

The skin of the albino does not tan after exposure to ultraviolet light The erythemal threshold of unexposed skin is lower than in normal white skin By carefully graded exposures the albino can achieve a fair degree of tolerance to solar exposure by a thickening of the stratum corneum



Fig 13-3 Dermal melanocytic nevus in generalized albinism (same patient as in Fig 13 2)

*Physiologic Responses of Albino Skin* There have been few if any studies of various physiologic responses of albino skin Information is needed on wound healing percutaneous absorption sensory function sweat and sebaceous gland secretion insensible water loss pH circulation and vascular reactions thermoregulatory mechanisms and electrical behavior At present the sole recognized abnormal property of albino skin is the absence of melanin pigment Albinos do not become pigmented during pregnancy and the nipples remain pink [21]

*Pigmented Lesions in Albino Skin* Albino skin contains melanocytes which can proliferate into benign nonpigmented and pigmented nevi [22] (Fig 13-3) There are three case reports of malignant melanomas arising in melanocytes of albino skin [23-25]

In addition to pigmented and nonpigmented melanocytic nevi large pigmented freckles (*ephelides*) may occur in the skin of some albinos

especially on the cheeks forehead extensor aspects of the arms neck and the upper region of the back and chest, but not on the legs [14, 26 *,] Exposure to sunlight may be a factor in their development Their clinical and histologic appearance as reported by Barnicot [14] is similar to that of ephelides in normal Caucasians

**Hair** *Color* The hair color in the Negro albino ranges from "white faintly tinged with yellow to a yellowish brown color ' [14] The hair is said to darken with age as in normal persons Microscopic examination of Negro albino hair reveals the presence of fine pigment granules in the cortex in the darker strands of hair [14 28] The hair of Caucasian albinos is often, but not always white and ranges through all shades of cream yellow yellow red and even strong red Thus the range of the hair color of the Caucasian albino merges with that of the normal blond Caucasian Indeed some extreme blond nonalbino hair is lighter than that of the true albino

Banding (alternating white and dark bands) has been observed in the African albino

*Texture* The texture of Caucasian albino hair is unusually fine but that of the Negro albino is unchanged [28]

**Eye** *Color* There is a common misconception regarding the iris color and pupillary reflex of generalized albinos which may be based on the pink eye of albino mice and rats While it is true that the iris color is a light gray and the pupil red in generalized albino *infants* the commonest finding in generalized adult albinos is a blue gray translucent iris a black pupil, and a decrease or absence of retinal and choroidal pigment The red pupil occasionally noted in generalized albino infants darkens to black with age

The iris color in Negro albinos ranges from a pale blue to a cinnamon brown [26] A range of light brown green blue green and blue was recorded by Barnicot [14] in African Negro albinos He observed only a single albino child with a red pupil reflex

The fundus is usually bright orange red with prominent choroidal and retinal vessels The macula is uniformly pink Falls [7] believes that a greater degree of pinkness is correlated with more efficient visual acuity The absence or poor development of the fovea centralis in the albino eye may be the chief cause of the defective vision [29]

Perhaps the clinical feature of the albino most obvious to both patient and physician is photophobia present even in the relatively darkly pigmented Negro albino iris The photophobia probably results from the ' dazzling introduced by the lack of contrast between the retinal impulse and the surrounding media

Visual acuity is almost always decreased in generalized albinism In a study of 16 albinos Edmunds [30] found an average visual acuity of 20/200 Nine were hypermetropic 5 myopic and 13 had astigmatism The

visual fields are abnormal and usually there is a central scotoma Color vision and dark adaptation are normal Strabismus (both divergent and convergent type ) is not uncommon

The albino eye almost always shows a horizontal nystagmus The poor central vision (due to central scotoma) allows the subcortical rhythm center normally held in check by the cortex to produce involuntary rhythmic oscillations [30] Congenital ocular abnormalities associated with albinism include absence of the sphincter muscle partial aniridia congenital pupillary membrane, atrophy of the disk and coloboma of the mesodermal tissue of the iris [31]

The albino eye is normal histologically except for the decrease or absence of pigment in the retinal pigment epithelium and choroid and hypoplasia or aplasia of the macular area

**Miscellaneous Abnormalities Associated with Generalized Albinism** Numerous abnormalities have been associated with generalized albinism These include deaf mutism epilepsy polydactyly and oligophrenia The incidence of these abnormalities has not been reported in a large series and the frequency of their occurrence is not known

The intelligence of generalized albinos is normal [31 32] but no large series has been studied The condition is compatible with unusual intelligence Sachs [33] was an albino scientist who chemically analyzed his own hair Reverend W A Spooner (1844–1930) a distinguished scholar Warden of New College Oxford University and of spoonerism fame was a generalized albino

**Localized Albinism of the Eye (Ocular Albinism)** There are two types of albinism limited to the eye In one there is absence of pigment in the retinal pigment epithelium In the other there is additionally a decreased quantity of iris pigment Both varieties have nystagmus and decreased visual acuity but normal pigmentation of the skin and hair

**Localized Albinism of the Skin and Hair (Partial Albinism)** Albinism may be localized to the skin and hair without eye involvement (Fig 13-4) This has been variously designated as albinoidism congenital achromia congenital leukoderma congenital vitiligo poikilochromia alba white spotting and piebaldness When limited to the hair alone it may be called poliosis circumscripta white forelock white blaze and partial albinism The essential features have been summarized by Cooke [34] Depigmentation of a tuft of hair in the midfrontal area (white forelock) is present in the majority of cases The loss of hair color may extend over one-half of the frontal area and may involve the eyebrows and eyelashes without involving the uveal tract or retinal pigment epithelium The depigmentation may extend to one half of the face to include the orbital infraorbital buccal and zygomatic regions

Unpigmented lesions of the trunk and extremities (which may be unilateral or bilateral) most commonly involve the abdomen and ventral

thorax The unpigmented areas of the face, trunk, or extremities may simulate the clinical picture of vitiligo

Unpigmented areas may partially repigment in the second and third decade A father may have almost completely *repigmented* areas on the abdomen whereas his son may show large white macular lesions in the same area [35]



Fig 13-4 Localized cutaneous albinism in two siblings Note the white forelock (*From H M Jahr et al [12] By permission of the American Medical Association*)

## BIOCHEMICAL LESION OF HUMAN ALBINISM

Speculations on the nature of albinism were made by Garrod [4] in 1908 when he attributed the absence of melanin to a *metabolic* defect of the pigment cell and in 1911 by Pearson [1] who maintained that albinism was a *structural* defect or an absence of pigment cells These conflicting views have been partially resolved in the past 50 years

Much progress has been made in knowledge of the genes in mice and guinea pigs which control the differentiation of pigment cells the size shape and color intensity of the melanin granule and the synthesis of the enzyme tyrosinase Four factors influence the activity and function of melanocytes in the mouse (1) the genotype of the melanoblast (2) the genotype of the environmental cells (3) the environmental history of the melanoblast and (4) the differentiated characteristics of the environmental cells [36] In man it has not been possible to identify the genes responsible for hair eye and skin color and to relate the various types of

albinism to specific gene loci but this has been done with some success in other species

It is now possible to give a fairly complete answer to the question of whether generalized albinism in man is a *structural* or a *metabolic* defect In the last decade techniques have been developed for study of the biochemistry and cytochemistry of melanogenesis and the fine structure of melanocytes Two basic questions must be answered for an understanding of the nature of albinism (1) Are melanocytes present in albino skin hair and eyes? (2) What is the evidence for a biochemical lesion in albinism?

### EVIDENCE FOR THE PRESENCE OF MELANOCYTES

The identification of amelanic melanocytes by stains is unsatisfactory In 1952 Becker et al [37] reported the identification of melanocytes in

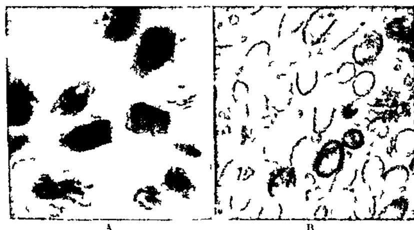



Fig 13-5 A High magnification (×30 000) field of forming melanin granules in a melanocyte from dark Caucasian hair B Same from an albino hair bulb Granules show varying stages of melanization in (A) and absence of melanization in (B) (*From* *A Barnicot et al* [33] *By permission of Academic Press Inc New York*)

human albino skin by the gold impregnation technique Silvers [38] has now shown by ingenious studies in mice that gold impregnated dendritic shaped cells are present in limb buds removed before the stage of migration of melanocytes from the neural crest into these structures Thus the branched cells revealed by gold chloride have no relation to melanocytes

The study of melanocytes with electron microscopy has provided a positive identification of amelanic melanocytes containing amelanic melanin granules [39] The melanocytes in the human albino hair bulb were identified by their cytoplasmic dendritic processes and were shown to contain in addition to mitochondria and Golgi material numerous ovoid or rod shaped bodies 0 ½μ or less in length (Fig 13-5) These latter

structures represent amelanic melanin granules These cells presumably have normal biochemical systems with the exception of the enzyme tyrosinase

The presence of melanocytes in albino skin is also suggested by the occurrence of benign melanocytic nevi and malignant melanoma in generalized albinism (Fig 13 3)

Finally, pigmentless melanocytes in generalized albinism are easily identified in the retinal pigment epithelium

On the basis of these facts it seems fairly certain that the structural basis for melanogenesis is present in generalized albinism There is no evidence which would allow one to consider generalized albinism a structural defect as proposed by Pearson [1]

## SITE OF THE METABOLIC BLOCK

*The biochemical lesion of albinism consists of a failure of melanocytes to synthesize tyrosinase a copper-containing oxidase required for the conversion of tyrosine to melanin*

Tyrosinase is synthesized and localized on the melanin granule the metabolic unit of melanogenesis The melanin granule is a spherical or rod shaped body from 0 1 to 0 5$\mu$ in diameter in the cytoplasm of the melanocyte It is distinctive from mitochondria In addition to tyrosinase melanin granules contain two other enzymes, succinic dehydrogenase and cytochrome oxidase [40]

The process of melanogenesis involves a conversion of tyrosine to a brown or black insoluble polymer (Fig 13 6) Tyrosinase catalyzes the first step, the hydroxylation of tyrosine to dihydroxyphenylalanine (dopa) The next step the oxidation of dopa to dopa quinone is markedly accelerated by tyrosinase but can occur nonenzymatically

The balance of the pathway may be nonenzymatic Dopa quinone cyclizes to 5 6-dihydroxyindole-2 carboxylic acid and then is oxidized to the corresponding quinone dopachrome Dopachrome by an internal oxidation reduction loses carbon dioxide to form 5 6-dihydroxyindole which is the immediate precursor of melanin The polymerization of the monomer 5 6-dihydroxyindole to a polymer (melanin) occurs by oxidation to indole 5 6-quinone and repeated condensations between the anionoid centers in the pyrrole ring of one molecule and cationoid centers in the quinonoid ring of adjacent molecules This gives rise to a polymer of high molecular weight [41] For this polymerization the 3 position and the 4 or 7 position must be free but the 2 3 4 and 7 positions all play a part in the building up of a three-dimensional polymer of high molecular weight [41] (Fig 13 7) The melanin polymer is attached to protein through its quinone linkages with amino or sulfhydryl groups of protein

Melanin pigment (in the melanocyte) seen in the light microscope as a

brown or black cytoplasmic inclusion is not an inert particle but a functioning metabolic unit containing a heterogeneous mixture of protein

TYROSINASE
Slow (0)
Fast (0)
TYROSINE
3,4 Dihydroxyphenylalanine
Dopa Quinone
Fast
Enzymatic or Nonenzymatic
Leuco Dopachrome
Enzymatic or Nonenzymatic Fast
Dopachrome (red)
Zn or A Slow
5,6 Dihydroxyindole
Fast
Enzymatic or Nonenzymatic
Indole 5 6 quinone (yellow)
Slow
MELANIN

Fig 13-C Metabolic pathway of tyr to mel n

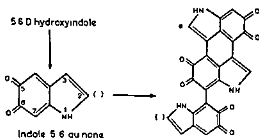



Fig 13-4 Suggested polymerization in melanin formation Asterisks indicate sites of coupling of monomers (After I I T Co tie et l ('1))

melano-protein tyrosinase and perhaps two other enzymes The elucidation of the structure of natural melanin is a challenging and difficult problem because of the heterogeneity of the melanin granule

Melanin is defined as a brown or black insoluble pigment contained on melanin granules in the cytoplasm of melanocytes and formed from tyrosine by tyrosinase The melanin in the cells of the melanocyte system

structures represent amelanic melanin granules The e cells presumably have normal biochemical systems with the exception of the enzyme tyrosinase

The presence of melanocytes in albino skin is also suggested by the occurrence of benign *melanocytic nevi and malignant melanoma in* generalized albinism (Fig 13 3)

Finally pigmentless melanocytes in generalized albinism are easily identified in the retinal pigment epithelium

On the basis of these facts it seems fairly certain that the structural basis for melanogenesis is pre ent in generalized albinism There is no evidence which would allow one to con ider generalized albinism a *structural defect, as proposed by Pearson [1]*

## SITE OF THE METABOLIC BLOCK

*The biochemical lesion of albinism consists of a failure of melanocytes to synthesize tyrosinase, a copper containing oxidase required for the conversion of tyrosine to melanin*

Tyrosina e is synthe ized and localized on the melanin granule the *metabolic unit of melanogenesis The melanin granule is a spherical or* rod shaped body from 0 1 to 0 5$\mu$ in diameter in the cytoplasm of the melanocyte It is distinctive from mitochondria In addition to tyrosinase melanin granules contain two other enzymes, succinic dehydrogenase and cytochrome oxidase [40]

The process of melanogenesis involves a conversion of tyrosine to a brown or black insoluble polymer (Fig 13 6) Tyrosinase catalyzes the first step the hydroxylation of tyrosine to dihydroxyphenylalanine (dopa) The next step the oxidation of dopa to dopa quinone is markedly accelerated by tyrosinase but can occur nonenzymatically

The balance of the pathway may be nonenzymatic Dopa quinone cyclizes to 5 6 dihydroxyindole 2 carboxylic acid and then is oxidized to the corresponding quinone dopachrome Dopachrome by an internal oxidation reduction loses carbon dioxide to form 5 6-dihydroxyindole which is the immediate precursor of melanin The polymerization of the monomer 5 6 dihydroxyindole to a polymer (melanin) occurs by oxida tion to indole 5 6-quinone and repeated condensations between the anionoid centers in the pyrrole ring of one molecule and cationoid centers in the quinonoid ring of adjacent molecules This gives rise to a polymer of high molecular weight [41] For this polymerization the 3 position and the 4 or 7 position must be free but the 2 3 4 and 7 positions all play a part in the building up of a three-dimensional polymer of high molecular weight [41] (Fig 13 7) The melanin polymer is attached to protein through its quinone linkages with amino or sulfhydryl groups of protein

Melanin pigment (in the melanocyte) seen in the light microscope as a

brown or black cytoplasmic inclusion is not an inert particle but a functioning metabolic unit containing a heterogeneous mixture of protein

TYROSINASE
Slow (O)
Fast (O)
TYROSINE
3,4 Dihydroxyphenylalanine
Dopa Quinone
Fast
Enzymatic or Nonenzymatic
Zn or A Slow
Enzymat c or Nonenzymatic Fast
5 6 D hyd o yindole
Dopachrome (red)
Leuco Dopachrome
Fast
Enzymat c or Nonenzymat c
Slow
MELANIN
Indole 5 6 qu none (yellow)

Fig 13-6 Metabolic patl way of tyrosin to melanin

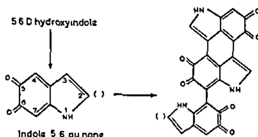



Fig 13-7 Suggested poly ner zat on in melanin formation Asterisks in l ate sites f co ipling of monomers (After R I T Crom lie et al [41])

melano protein tyrosinase and perhaps two other enzymes The elucida tion of the structure of natural melanin is a challenging and difficult problem because of the heterogeneity of the melanin granule

Melanin is defined as a brown or black insoluble pigment contained on melanin granules in the cytoplasm of melanocytes and formed from tyrosine by tyrosinase The melanin in the cells of the melanocyte system

is included in this definition because the melanin pigment is contained in melanocytes and the tyrosine tyrosinase system occurs in the melanocytes of the skin [12] hair bulb [13] retinal pigment epithelium [5] and uveal tract [40] Melanocytes in the pia-arachnoid have not yet been studied for tyrosine tyrosinase activity, but it is probable that they are part of the melanocyte system because of the origin of primary malignant melanoma in the pia-arachnoid

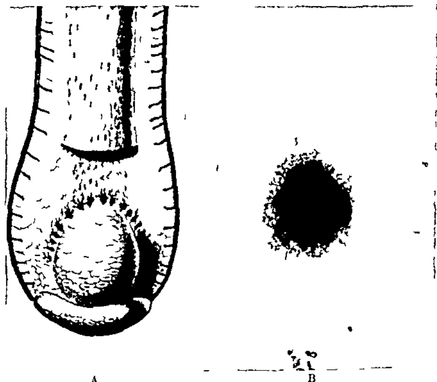



Fig 13 8 Normal human black hair A The melanocytes occupy the upper portion of the hair matrix B Radioautographic tyrosinase method Lithium carmine Magnification ×320 The tyrosinase contained in the hair bulb melanocytes has catalyzed the oxidation of $C^{14}$ labeled tyrosine to $C^{14}$ labeled melanin The sites of radioactive melanin are represented by dense masses of silver grains Tissue was incubated in vitro in 0 3 microcurie of DL-tyrosine 2 $C^{14}$

The nature of the pigment in the substantia nigra and the locus caeruleus has not been clarified The pigment in these areas is unchanged in generalized albinism [44] and this would suggest that it is elaborated by some other pathway than by the action of tyrosinase Malignant melanomas or melanocytic nevi do not arise from the pigmented cells of the substantia nigra and locus caeruleus However these data are not

sufficient to conclude definitely that the pigment is not tyrosine-melanin derived from tyrosine by the action of tyrosinase Further studies are necessary especially to identify tyrosinase in these cells Fellman [45] has proposed that the pigment in these two areas arises from the oxidative polymerization of epinephrine or norepinephrine

Manometric measurement of the tyrosinase in certain alleles of the albino series of mice (C series) has revealed a complete absence of tyrosinase activity [46] In man it is not feasible to obtain large enough quantities of melanocytes from the hair bulb or skin for manometric estimation of tyrosinase




Fig 13-9 Albino hair bulbs of man (A) and mouse (B) genotype (a/a B/B c/c D/D P/P) Radioautographs tyrosinase method Compare with Fig 13-8 The virtual absence of silver grains is indicative of the lack of tyrosinase in the melanocytes of the albino hair bulbs of man and mouse genotype

The use of histochemical procedures for tyrosinase detection has proved highly satisfactory and rewarding The available methods are more sensitive than manometry are equally specific and have the added advantage of localizing precisely the enzyme activity in the melanocytes Most of the histochemical studies of melanin formation in the past have utilized dopa the second product in the oxidation of tyrosine to melanin as substrate This has serious limitations for dopa is unstable and is nonspecifically oxidized to melanin by a variety of enzymes and oxidizing systems other than tyrosinase

The development of radioautography utilizing $C^{14}$ labeled tyrosine as substrate has provided a relatively simple method for detection of tyrosinase activity [43 47 48] This method now fairly well standardized is highly specific and more sensitive than either manometric methods or histochemical procedures with nonradioactive substrates

Figure 13 8 illustrates the demonstration of tyrosinase with the histochemical radioautographic technique Tyrosinase cannot be detected in

the melanocytes of the skin or hair bulb of the human generalized albino The absence of tyrosinase in the hair bulb of the human generalized albino and the albino mouse of specific genotype is shown in Fig 13 9

Tyrosinase activity in the human epidermal melanocyte is of such low concentration (or is present in an inhibited form) that it cannot be demonstrated unless the skin is previously irradiated in vivo [42] When ultraviolet irradiated skin slices are incubated in tyrosine melanin readily appears in the melanocyte as brown granules in the perikaryon and cytoplasmic processes

The failure of melanin formation from the melanogenic substrates tyrosine and dopa in albino melanocytes could hypothetically result from a lack of machinery in the melanocyte (1) for tyrosinase synthesis (2) for melanin polymerization or (3) for synthesis of the precise protein of the

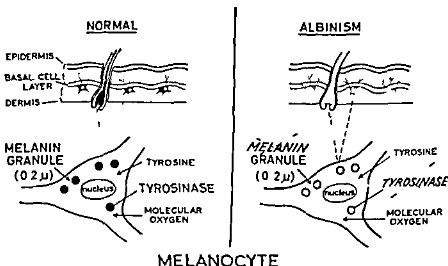



Fig 13 10 The biochemical lesion in albinism

melanin granule Since amelanic pigment granules in the human albino hair bulb appear identical to the melanic pigment granules of the normal hair bulb when viewed in the electron microscope it would appear that the metabolic defect in albinism is a failure either of tyrosinase synthesis or of melanin polymerization The latter possibility is precluded by the absence of oxygen consumption in preparations of fetal albino guinea pig skin in the presence of tyrosine [49] This indicates a failure of hydroxylation and dehydrogenation If the defect in albinism were simply a failure of the monomer indole 5 6 quinone to polymerize to melanin oxygen consumption as required for the formation of the monomer would be unimpaired Thus from all the available evidence it can be concluded that the biochemical lesion in albinism is an absence of the gene in the

melanocyte which is required for the synthesis of tyrosinase in the melanin granule (Fig 13 10)

### EFFECT OF THE ABSENCE OF TYROSINASE ON AROMATIC AMINO ACID METABOLISM

In phenylketonuria where decreased melanin pigmentation of the hair [50] and iris [51] is found there is a marked accumulation of phenylalanine and related metabolites in the extracellular fluids as a result of the reduced amount or complete absence of L phenylalanine oxidase [52] (cf Chap 10)

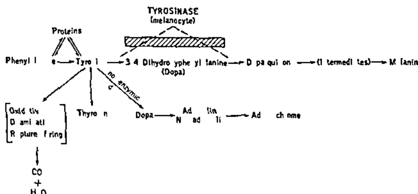



Fig 13-11 Intermediary metabolism of phenylalanine and tyrosine and site of metabolic block in albinism

The accumulated phenylalanine and its metabolites perhaps acting as competitive inhibitors cause (1) inhibition of tyrosinase [53 54] resulting in a decrease in hair and iris color (2) inhibition of the incorporation of tyrosine into proteins of liver slices [55] (3) an accumulation of phenylalanine and tyrosine in the adrenal gland and reduced amounts of norepinephine and epinephine [45] and (4) a decreased amount of pigment in the substantia nigra and locus caeruleus [45] A parallel effect of accumulated metabolites in albinism on the other aromatic pathways may be found but this problem has not been studied It will be necessary first to determine the concentration of tyrosine and related metabolites in the extracellular fluids of albinos The content of pressor amines in the urine of a generalized albino was within normal limits [56] It is probable that tyrosine is hydroxylated to dopa in the adrenal gland by another pathway not requiring tyrosinase This would explain the normal amounts of pressor amines in albinos who lack tyrosinase in melanocytes and cannot hydroxylate tyrosine to dopa in these cells (Fig 13 11)

In order to conclude that a particular metabolic disorder is a consequence of a deficiency of a certain enzyme two types of evidence are required [57]

1 An in vitro demonstration of a decrease or absence of the enzyme with certainty that the failure to detect the enzyme is not due to the presence of inhibitors or some other environmental factor

2 Demonstration of biochemical changes in body fluids and tissues which can be attributed to the failure of a particular reaction normally catalyzed by the enzyme

The biochemical and histochemical studies described in this review appear to satisfy the first requirement of the in vitro demonstration of an absence of tyrosinase in the melanocytes The second postulate is satisfied by the decrease or absence of melanin formation in the skin, hair, and eyes which is the direct result of the absence of tyrosinase which catalyzes the tyrosine-melanin pathway

## GENETICS

Both universal and generalized albinism are inherited as autosomal recessive traits This interpretation is based on more than 700 pedigrees reported in the past 30 years Hogben s [58] analysis of Pearson s data *consisting of over 600 pedigrees is the most complete available* Pipkin and Pipkin [26] in a study of the pedigrees of 5 albino families concluded that 3 were autosomal recessive and 2 autosomal dominant An excellent recent study of 122 albinos resident in Northern Ireland [2] supports an autosomal recessive inheritance In Froggatt's study [2] the consanguinity rate for first cousin matings was 4 49 per cent Higher consanguinity rates have been reported by Pearson [1] (11 to 14 per cent) and Sanders [59] (10 7 per cent) A loose linkage of the genes for albinism and sicklemia is suggested by the observation of a Negro family in which albinism and sicklemia occurred [60] One albino was homozygous for the sickle cell gene Wright (quoted by Castle [61]) suggested that albinism in man may be inherited as a graded series of allelomorphs similar to the guinea pig series Knox has commented on the occurrence of albinism on the basis of a series of allelic as well as nonallelic genes He cites as evidence the instance of two normally pigmented infants with good vision born from the union of two generalized albinos [64] Knox also cites the existence of *rufous* albinism or "xanthism " as suggestive of allelic genes Although xanthism has not been directly linked with generalized albinism Loewenthal [63] has reported the occurrence of nystagmus and freckling (two characteristic features of generalized albinism) in two African negroes with xanthism A discussion of the genetic history of albinism is included in Knox s [64] excellent review

Localized albinism of the skin (partial albinism) is inherited as a dominant gene with high penetrance [12 35 59] In a complete summary of the literature Cooke [34] collected 23 families She found that when one parent had the trait more than half (53 2 per cent) of the 769 children

were affected while of more than 474 children born of unaffected parents only 15 (3 1 per cent) inherited the trait The various discrepancies in the transmission of localized cutaneous albinism may be explained by lack of recognition of the trait because of fair skins in those subjects in whom the characteristic white forelock is absent In addition localized cutaneous albinism is easily confused with vitiligo localized to one area

Localized cutaneous albinism has been known to occur in the same pedigree as generalized albinism While the two conditions are presumably not related to each other they are both genetically transmitted

Localized pigmentary loss in the hair of mice (known as spotting) is related to the action of genes at seven different loci In this anomaly there is a *loss of melanocytes* from the hair follicles of the spotted regions The pigmentary defect is not the result of gene-enzyme deficiency as in albinism [65] Since there have been no studies of localized (partial) albinism to determine the presence or absence of melanocytes one cannot state for sure whether localized albinism in man is a metabolic (tyrosinase) defect or a structural (melanocyte) defect

Localized albinism of the eye is inherited as an X chromosomal (sex linked) trait Waardenburg [66] observed iris translucency in the hetero zygote or carrier female and Falls [67] described a characteristic fundus in the female heterozygote This pigmentary change consists of small islets of coarse cocoa brown pigment clusters and stippling of the macular areas These subjects have normal vision Thus Waardenburg s and Falls observations suggest an intermediate inheritance pattern in which there is limited expression in the heterozygous condition

## SUMMARY

1 Albinism is an inherited metabolic defect of the melanocyte in which that cell fails to form melanin The metabolic defect may involve the entire melanocyte system (universal albinism) the majority of the melanocytes in the melanocyte system (generalized albinism) or only one locus of melanocyte (localized albinism of the skin or eye)

2 Universal albinism has been observed in almost all species Generalized albinism in man occurs in a ratio of from 1 5 000 to 1 25 000

3 Pigmentless melanocytes are present in the albino Amelanic melanin granules can be visualized in the albino hair bulb with the aid of the electron microscope

4 The biochemical lesion in albinism is a failure of the melanocyte to synthesize tyrosinase This enzyme is required for melanin formation in the melanocyte The enzyme defect has been established by a histochemical radioautographic technique utilizing $C^{14}$ labeled tyrosine as substrate

5 The absence of tyrosinase in the melanocyte system may have an

effect on other aromatic amino acid pathways but thus far there has been little or no investigation of possible accumulated metabolites in albinism The formation of epinephrine and norepinephrine does not appear to be affected in the albino

## BIBLIOGRAPHY


1 Pearson K Nettleship E and Usher C H *A Monograph on Albinism in Man (6 vols) series VI VIII and IX parts I II and IV* Cambridge London 1911–1913
2 Froggatt P Thesis presented for the degree of Doctor of Medicine Trinity College Dublin 1957
3 Wafer L *A New Voyage and Description of the Isthmus of America Giving an Account of the Author's Abode There Collection of Voyages* 3d ed vol 3 pp 261–463 London 1729
4 Garrod A E Inborn errors of metabolism Lecture I Lancet 2 73 1908
5 Miyamoto M and Fitzpatrick T B On the nature of the pigment in retinal pigmented epithelium Science 126 449 1957
6 Klein D Metabolic disorders in *Clinical Genetics* edited by A Sorsby p 181 Butterworth London 1953
7 Falls H F Albinism Tr Am Acad Ophth 57 324 1953
8 Shufeldt R W Albinism in American animals Proc Zool Soc London 540 1916
9 Little C C Coat color genes in rodents and carnivores Quart Rev Biol 33 103 1958
10 Robinson R Partial albinism in the Syrian hamster Nature London 180 443 1957
11 Stout D B Albinism among San Blas Cuna Panama further notes Am J Phys Anthropol 4 483 1946
12 Jahr H M and McIntire M S Piebaldness or familial white skin spotting (partial albinism) A M A Am J Dis Child 88 481 1954
13 Daniels F Jr Personal communication 1958
14 Barnicot N A Albinism in South Western Nigeria Ann Eugenics 17 38 1952
15 Barnicot N A Human pigmentation Man 57 114 1957
16 *Shapiro M P Keen P Cohen L and Murray J F Skin cancer in the South African Bantu* Brit J Cancer 7 45 1953
17 MacDonald E Epidemiology of skin cancer J Invest Dermat 33 379 1959
18 Blum H F Sunburn in *Radiation Biology* edited by A Hollaender vol 2 p 487 McGraw Hill New York 1955
19 Pathak M Daniels F Jr Hopkins C E and Fitzpatrick T B Ultraviolet carcinogenesis in albino and pigmented mice receiving furocoumarins psoralen and 8-methoxypsoralen Nature London 183 728 1958
20 Thomson M L Relative efficiency of pigment and horny layer thickness in protecting the skin of Europeans and Africans against solar ultraviolet radiation J Physiol 127 236 1955
21 Richardson B W Mammary areola in the pregnant albino Dublin Hosp Gaz 3 73 1856
22 Ito M Lentigo achromiant in albinos Ann dermat et syph 83 631 1956
23 Bhende Y M Malignant amelanotic melanoma of the skin in an albino Indian J M Sc 6 755 1952
24 Young T L Malignant melanoma in an albino A M A Arch Path 64 186 1958
25 Leonardi R and Crasso S Melanoblastoma in albino histological findings Minerva dermat 33 21 1958
26 Pipkin A C and Pipkin S B Albinism in Negroes J Hered 33 419 1942
27 Ales H The Indians of Panama—their physical relation to the Mayas Am J Phys Anthropol 9 1 1926
28 Pipkin S B and Pipkin A C Hair of Negro albinos J Hered 35 201 1944

29 Usher C H Histological examination of an adult human albino s eyeball with a note on me oblastic pigmentation in foetal eyes Biometrika 13 46 1920 19 1
30 Edmunds R T Vision of albino Arch Ophth 42 755 1949
31 Wallner A and Rudens M C Report on an unusual family Am J Ophth 33 785 1950
32 Beckham A S Albinism in Negro children J Genet Psychol 66 199 1946
33 Sachs G T L Historia naturalis duorum leucaetl iopum auctoris ipsius et sororis ejus 8th ed Solisbaci 181? (Quoted in ref 1)
34 Cooke J V Familial wl ite skin spotting (piebaldness) ( partial albinism ) with white forelock J Pediat 41 1 195?
35 Sanders J Two families with albinismus circumscriptus Genetica 17 185 1935
36 Markert C L and Silvers W K The effects of genotype and cell environment on melanoblast differentiation in the house mou e Genetics 41 429 1956
37 Becker S W Jr Fitzpatrick T B and Montgomery H Human melanogenesis cytology of human pigment cells (melanodendrocytes) A M A Arch Dermat & Syph 65 511 1952
38 Silvers W K A histological and experimental approach to determine the relationship between gold impregnated dendritic cells and melanocytes Am J Anat 100 2 5 1957
39 Barnicot N A and Birbeck M S C The electron microscopy of human melanocytes and melanin granules in *The Biology of Hair G owth* edited by W Montagna and R A Ellis Academic I res Inc New York 1958
40 Herrmann H and Boss M B Dopa oxidase activity in extracts from ciliary body and in isolated pigment granules J Cell & Comp Physiol 26 131 1945
41 Cromartie R I T and Harley Mason J Melanin and its precursors 8 The oxidation of methylated 5 6-dihydroxyindoles Biochem J 66 713 1957
42 Fitzpatrick T B Becker S W Jr Lerner A B and Montgomery H Tyrosinase in human skin demonstration of its presence and its role in human melanin formation Science 112 2 3 1950
43 Kukita A and Fitzpatrick T B Demonstration of tyrosinase in melanocytes of the human l air matrix by autoradiography Science 121 893 1955
44 Foley J H and Baxter D On the nature of pigment granules in tl e cells of the locus caeruleus and substantia nigra J Neuropath 17 586 1958
45 Fellman J H Epinepl rine metabolites and pigmentation in the central nervous system in a case of phenylpyruvic oligophrenia J Neurol Psychiat 21 58 1958
46 Foster M Inherited variations in the pigmentary metabol sm of mouse skin Genet cs 41 643 1956*B*
47 Fitzpatrick T B and Kukita A A histochemical autoradiographic method for demonstration of tyrosinase in human melanocytes nevi and malignant melanoma *J Invest Dermat* 26 173 1956
48 Fitzpatrick T B and Kukita A Tyrosinase activity in vertebrate melanocytes in *Pigment Cell Biology* edited l y M Go don Academic I ress Inc N w Yo k 1959
49 Foster M Inherited variations in the tyrosinase system in gu nea pig skin Genetics 37 581 195?*B*
50 Shizume K Unpublished data
51 Berg J M and Stern J Iris color in phenylketonuria Ann Human Genet 22 370 1958
52 Dalgl esh C E Metabolism of the aromatic amino acids Advances Protein Chem 10 31 1955
53 Dancis J and Bal s M E A poss l le mechanism fo distu bance in tyrosine metabolism in phenylpyruvic ol gophrenia I ediatrics 15 63 1955
54 Miyamoto M and Fitzpatrick T B Competitive inhibition of mammalian tyrosinase by phenylalanine and its relationsl ip to l air p gmentat on in phenyl ketonuria Nature London 179 199 1957
55 B ckis I J Kennedy J C and Quastel J H Phenylalanine inh bition of tyrosine metaboli m in the liver Nature Londo 179 11 4 1957
56 Fellman J H I ersonal comn unication

57 Harris H Genes and enzymes in man in *Novant anni delle leggi mendeliane* edited by L Gedda Instituto Gregorio Mendel Rome 1956
58 Hogben L T The genetic analysis of familial traits J Genet 25 97 1931
59 Sanders J Family with albinismus circumscriptus Genetica 16 435 1934
60 Massie R W and Hartman R C Albinism and sicklemia in a Negro family Am J Human Genet 9 127 1957
61 Wright S quoted by W E Castle Siamese an albinistic color variation in cats Am Naturalist 53 265 1919
62 Trevor Roper P D Marriage of two complete albinos with normally pigmented offspring Brit J Dermat 36 107 1952
63 Loewenthal L J A Partial albinism and nystagmus in Negroes 2 cases Arch Dermat & Syph 50 300 1944
64 Knox W E Sir Archibald Garrod's Inborn Errors of Metabolism III Albinism Am J Human Genet 10 249 1958
65 Silvers W K Pigment cells occurrence in hair follicles J Morph 99 41 1956
66 Waardenburg P J Herkenbaarheid van latente overdragers van albinismus universalis en albinismus oculi Ned Tijdschr Geneesk 91 1863 1947
67 Falls H F Sex linked ocular albinism displaying typical fundus changes in the female heterozygote Am J Ophth 34 41 1951

# Chapter 14

# Primary Hyperoxaluria and Oxalosis*

*James B. Wyngaarden and T. David Elder*

## DEFINITIONS

*Primary hyperoxaluria* is a rare disorder characterized clinically by progressive bilateral calcium oxalate urolithiasis and nephrocalcinosis beginning in early childhood and by death in childhood or early adult life from renal failure. Recurrent infection of the urinary tract and hypertension are common secondary features. The disorder is characterized biochemically by a continuous high urinary oxalate excretion, which is thought to result from a genetically controlled metabolic defect leading to overproduction of oxalate. The additional diagnosis *oxalosis* signifies the presence of disseminated extrarenal calcium oxalate deposits, which may develop as a stage in the natural history of primary hyperoxaluria.

## HISTORICAL INTRODUCTION

Calcium oxalate crystals were first identified in urine by Donné in 1838. In the following years the medical literature accumulated many reports in which attempts were made to correlate diverse clinical signs and symptoms with the excretion of oxalate crystals. This endeavor gradually declined when it was appreciated that calcium oxalate crystals are normal constituents of urine whose formation is dependent upon the pH of the urine and the concentration of other solutes. A voluminous and often uncritical literature dealing with oxalate metabolism was extensively reviewed in 1945 by Jeghers and Murphy [1].

The first reported case of oxalosis is probably that of Lepoutre [2], who in 1925 briefly described a 4½ year-old child with bilateral urolithiasis in whom a renal biopsy showed extensive crystalline deposits of

* The authors' studies were supported by a grant (A 1391) from the Public Health Service.

calcium oxalate In 1950, Davis Klingberg and Stowell [3] reported the case of a 12-year-old boy in whom renal calculus formation nephrocalcinosis, and deposition of calcium oxalate in extrarenal tissues were demonstrated They suggested the possibility of an underlying metabolic defect In 1952 Chou and Donohue [4] published a similar case and first employed the term "oxalosis" The first recorded observation of hyperoxaluria in a patient with probable oxalosis is that of Newns and Black (1953) [5] but histologic demonstration of oxalate deposits in tissues was not obtained In 1954 Aponte and Fetter [6] described three cases of oxalate nephrocalcinosis occurring in one family All were boys and the first two were identical twins Death was due to renal failure at 16 13½ and 9 years respectively The twins had urinary oxalate excretions of 200 and 180 mg per 24 hr and both showed oxalate deposits in vertebral marrow at autopsy These two cases are the first definite examples of oxalosis in which all critical criteria are fulfilled

## CLINICAL REVIEW

About thirty case reports have been published which suggest the diagnosis of oxalosis, but only 19 of these may be considered reasonably definite examples on the basis of early age of onset and demonstration of hyperoxaluria or extrarenal deposits of calcium oxalate or both [3–15, 17–19] The major features of these 19 cases and of one new case are summarized in Table 14 1

The age at onset ranged from 15 months to 17 years, but the majority of patients at onset were in the 1 to 4 year range The majority of the patients were males and all subjects in whom race was recorded were white In 16 fatal cases the duration of the illness from onset of symptoms ranged from 1 month to 18 years with a mean of 4 years

Although the disease is generally characterized by repeated attacks of renal colic hematuria and passage of calculi 4 cases presented in uremia without this history [6 7] The diagnosis was established prior to death in 5 cases [5 11 17 19] In each multiple pure calcium oxalate stones were passed many calcium oxalate crystals were found in the urine and the 24 hr urinary excretion of oxalate was elevated Nephrocalcinosis was diagnosed by x ray in 3 of these cases and by biopsy in a fourth oxalosis was established in 1 by examination of bone marrow [19]

### PHYSICAL FINDINGS

Physical examination reveals nothing characteristic of this disease In spite of chronic renal disease the blood pressure is frequently normal

The eyes have been examined carefully for calcium oxalate crystals in at least three cases [11 17] but thus far none has been found although they occur in other disorders [20 21]

The kidneys have not been palpably enlarged and no soft tissue calcifications have been described

## LABORATORY AND X RAY FINDINGS

A plain x ray film of the abdomen will usually reveal the presence of dense bilateral renal calculi which may frequently be recognized as calcium oxalate stones by their characteristic crystalline structure with spicules radiating from a central point They are usually triangular or jackstone in shape [22] Stones are also frequently observed in the ureters and bladder

As the disease progresses small focal calcifications appear in the area of the kidney parenchyma opposite the second and third lumbar vertebrae Radiographic signs of nephrocalcinosis are usually limited to the medullary regions but in two cases [8 10] the entire kidney was diffusely involved There was also extensive calcification of ureters and renal pelves in one case reported by Aponte and Fetter [6]

Skeletal x rays were normal in 7 of 9 patients In 2 [8 9] changes consistent with renal osteodystrophy were found Similar x rays were published by Caffey [22a] from a patient with oxalosis whose case has not been described in detail

Serum calcium levels above 10 5 mg per 100 ml were reported in 8 cases but remained consistently elevated in only 3 [3 11] In each of these instances 24 hr urinary calcium excretion was normal or low The serum phosphorus level was normal in all determinations until renal damage resulted in phosphorus retention Serum alkaline phosphatase level was also normal There is little reason to implicate parathyroid hyperfunction in these patients except as a secondary feature accompanying renal failure

The urine frequently showed pyuria hematuria and proteinuria and calcium oxalate crystals were often noted Urinary amino acid excretion was normal in 4 patients [5 9 11] Cystine was not increased in the urine of 3 subjects in whom it was measured [5 6]

## PATHOLOGY

On gross examination the kidneys are usually small with thickened capsules which strip with difficulty The surfaces are granular and contain focal depressed scars They are usually cut with noticeably increased resistance and in most cases a gritty sensation is present as though one were cutting through sand On the cut surface the cortex is thin and the pelves frequently contain calculi Small crystals may sometimes be seen in the parenchyma with the naked eye

On microscopic examination interstitial fibrosis and interstitial nephritis may be present Refractile crystals of various sizes are seen in the renal

tubules which may have compressed or destroyed the tubular epithelium (Fig 14 1) The crystals may extend into the interstitial spaces and have been described in the tunica media and adventitia of small renal arteries and arterioles The glomeruli are usually normal except for some moderate pericapsular fibrosis and a rare hyalinized glomerulus Extrarenal deposits

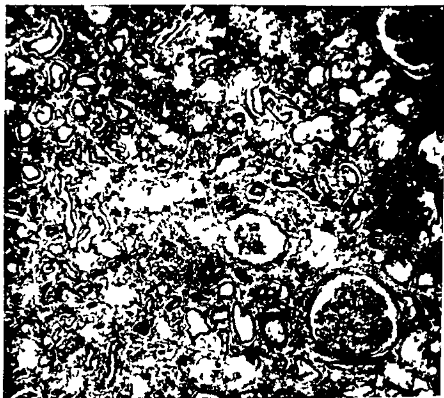


Fig 14 1 Oxalate nephrocalcinosis from a patient with primary hyperoxaluria and oxalosis Most of the calcium oxalate crystals are related to the remains of renal tubules (Half-crossed Nicol prisms × 70) *(From E F Scowen et al [15] with permission of the authors and publisher )*

of crystals have frequently been described in the bone marrow (Fig 14 2) *cartilage* (Fig 14 3) myocardium thyroid spleen liver lungs thymus pituitary adrenal testes pancreas parathyroids lymph nodes, and in the walls of veins arteries and arterioles [*8–19*]

Microscopically the crystals appear round and globular or rhomboidal in shape and have a radial rosette like pattern They have a slightly yellowish tinge and are doubly refractile under polarized light They do not stain with hematoxylin and eosin In most cases they do not stain with special techniques such as that of von Kossa The few instances in which the crystals did stain black with von Kossa s stain [*9–11 16*]



Fig 14- Calcium oxalate crystals in vertebral bone marrow from a patient with oxalosis (X 150) (*From H G Dunn [9] with permission of the author and publisher*)



Fig 14-3 Calcium oxalate crystals in costochondral cartilage of a patient with primary hyperoxaluria and oxalosis visualized under partial polarization (X 1"5) (*From J T Godwin et al [17] with permission of authors and publisher*)

may have been due to the presence of calcium carbonate or phosphate which was coprecipitated with oxalate [8]

The crystals may be identified in histologic sections as calcium oxalate by various chemical techniques The crystals are soluble when unstained deparaffinized sections are treated with concentrated hydrochloric or sulfuric acid and insoluble with lithium carbonate glacial acetic acid

tubules which may have compressed or destroyed the tubular epithelium (Fig 14-1) The crystals may extend into the interstitial spaces and have been described in the tunica media and adventitia of small renal arteries and arterioles The glomeruli are usually normal except for some moderate pericapsular fibrosis and a rare hyalinized glomerulus Extrarenal deposits

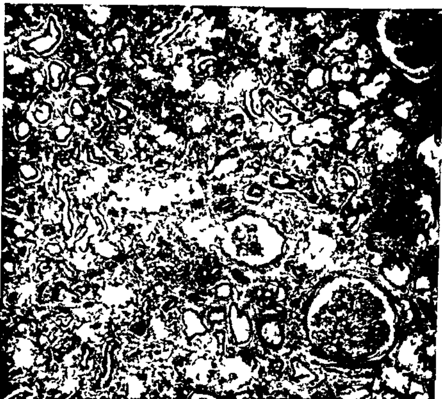


Fig 14 1 Oxalate nephrocalcinosis from a patient with primary hyperoxaluria and oxalosis Most of the calcium oxalate crystals are related to the remains of renal tubules (Half-crossed Nicol prisms × 70) *(From E F Scowen et al [13] with permission of the authors and publisher)*

of crystals have frequently been described in the bone marrow (Fig 14 2) cartilage (Fig 14 3) myocardium thyroid spleen liver lungs thymus pituitary adrenal testes pancreas parathyroids lymph nodes and in the walls of veins arteries and arterioles [*3*–19]

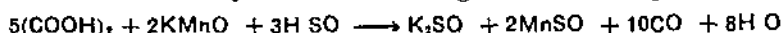
Microscopically the crystals appear round and globular or rhomboidal in shape and have a radial rosette like pattern They have a slightly yellowish tinge and are doubly refractile under polarized light They do not stain with hematoxylin and eosin In most cases they do not stain with special techniques such as that of von Kossa The few instances in which the crystals did stain black with von Kossa s stain [*9*–*11* *16*]

Oxalic acid is a relatively strong acid

$$K = 6.5 \times 10^{-2}$$
$$K = 6.1 \times 10^{-5}$$

It forms both acid and neutral salts mono and diesters a monoamide known as oxamic acid and a diamide known as oxamide

Oxalic acid is easily oxidized by ferric compounds and potassium permanganate and the latter compound is often used in quantitative determinations of oxalate by titration according to the following reaction



$$5(COOH)_2 + 2KMnO_4 + 3H_2SO_4 \longrightarrow K_2SO_4 + 2MnSO_4 + 10CO_2 + 8H_2O$$

In neutral or alkaline pH oxalate forms a calcium salt of very low solubility The precipitation of calcium oxalate from solution is inhibited by the presence of urea various ions such as sulfate and magnesium [27–29] and probably certain colloids [30] Under physiologic conditions urine is frequently supersaturated with calcium oxalate [27 31]

## BIOSYNTHESIS OF OXALIC ACID IN MAMMALIAN SYSTEMS

In mammalian systems the only known direct precursor of oxalic acid is glyoxylic acid Glyoxylic acid can arise from several sources the best known of which is glycine

### GLYCINE PATHWAY

Although the glycine pathway was postulated by Dakin as early as 1922 [31] the first demonstration that glycine serves as a precursor of oxalic acid was by Ratner Nocito and Green in 1944 [32] They prepared from a variety of mammalian kidney and liver sources an enzyme which oxidizes glycine (or sarcosine) to glyoxylic acid in the presence of flavin adenine dinucleotide (FAD) They named this enzyme glycine oxidase They also found an enzyme system in rat kidney and liver and cat muscle which catalyzes the conversion of glyoxylic acid to oxalic acid They believed this enzyme to be a mutase of the general type



2 glyoxylate ⟶ oxalate + glycolate

Although milk xanthine oxidase (aldehyde oxidase) also catalyzed the oxidation of glyoxylate to oxalate the presence of a second system in these tissues was indicated by the finding that the reaction proceeded anaerobically Kidney tissue appears to be the richest source of both glycine oxidase and mutase

A second reaction by which glycine may be converted to glyoxylic acid is that of transamination between glycine and $\alpha$ ketoglutaric acid [33] This reaction is freely reversible

Weinhouse and Friedmann [34] showed that glycine $C^{14}$ administered intraperitoneally in rats was extensively converted to glyoxylic acid and

concentrated ammonium hydroxide or concentrated potassium hydroxide When the crystals are dissolved in concentrated sulfuric acid small needle like crystals of calcium sulfate may occasionally form [16] If paraffin sections of tissue are first incinerated at 450 C for 30 min calcium oxalate is oxidized to the carbonate and small bubbles of carbon dioxide may be seen microscopically when concentrated sulfuric acid is allowed to run under the cover slip [23]

The crystals may also be identified as the monohydrate of calcium oxalate by optical examination The average width of single-needle crystals *is 0 005 mm and the average length is 0 05 mm They have a* birefringence of about 0 15 and an extinction angle of approximately 30° with respect to the length of the crystal The $\alpha$ and $\gamma$ indices of refraction are 1 49 and 1 65 by the immersion method The crystals have also been identified by x ray diffraction in several cases [9 10 16 17, 19]

Pathologically, the lesions are similar to those produced by experimental oxalate poisoning in animals in which the renal tubules are filled with calcium oxalate crystals compressing and causing necrosis of tubular epithelium [24-26]

## TISSUE OXALATE ANALYSES

Selected tissues were collected post mortem from a case of primary hyperoxaluria by Scowen Stansfield and Watts [13] and analyzed for calcium and oxalate contents The kidneys contained 67 2 and 65 0 mg $(COOH)_2 \cdot 2H_2O$ per gm dry weight the heart 1 19 mg $(COOH)_2 \cdot 2H_2O$ per gm dry weight Control tissue from four patients dying of unrelated illnesses contained from 0 to 0 06 mg $(COOH)_2 \cdot 2H_2O$ per gm dry weight Oxalate values of skeletal muscle and liver were not elevated in the hyperoxaluric subject and were 0 03 to 0 04 mg $(COOH)_2 \cdot 2H_2O$ per gm dry weight respectively Calcium values were correspondingly high in kidney and heart and were not appreciably elevated in skeletal muscle *and liver*

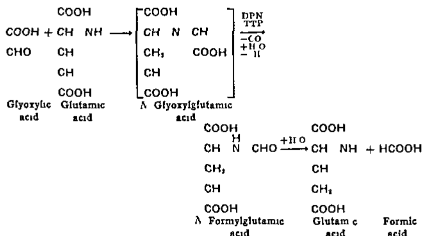
High concentrations of oxalate were present in the cerebrospinal and pleural fluids collected post mortem from the patient with primary hyperoxaluria No oxalate was detected in the cerebrospinal fluid from any of five 'control subjects including one who died from uremia due to malignant hypertension

## OXALIC ACID

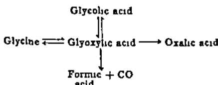
Oxalic acid is a dicarboxylic acid HOOC—COOH and may be crystallized as the white dihydrate The hydrate loses its water at about 100 C and the anhydrous acid sublimes with decomposition above 150 C It is soluble to the extent of 8 7 gm per 100 gm water at 20 C

*Glyoxylic Acid*

In addition to its reduction to glycolic acid its transamination to glycine and its direct oxidation to oxalate glyoxylate may be cleaved oxidatively to yield $CO_2$ and formic acid Nakada and Sund [39] have described an enzyme system tentatively designated glyoxylic acid dehydrogenase from rat liver mitochondria which requires diphosphopyridine nucleotide (DPN) thiamine pyrophosphate (TPP) and manganese ($Mn^{++}$) plus a stoichiometric quantity of L-glutamic acid Because of the requirement for glutamic acid and the isolation of the intermediate *N* formylglutamic acid they suggested that the process involves an initial condensation of glyoxylic acid and L-glutamic acid to yield a hypothetic intermediate *N* glyoxylglutamic acid which is then converted to $CO_2$ and N formylglutamic acid by the TPP DPN–dependent enzyme *glyoxylic acid dehydrogenase The formyl derivative may then enter* transformylation reactions or proceed by enzymatic hydrolysis to glutamic and formic acids

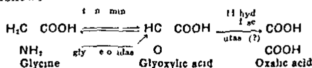



The data of Nakada and Weinhouse suggest that the major fate of glyoxylic acid is normally transamination to glycine a function that requires pyridoxal phosphate as coenzyme Reactions involving glyoxylic acid and oxalate formation are summarized below



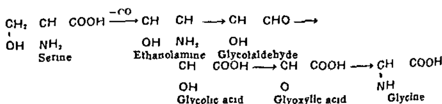
carbon dioxide and sparingly converted to oxalic acid Glyoxylic acid $C^{14}$ was extensively converted to glycine (isolated as hippuric acid) and oxalic acid but only sparingly oxidized to $CO_2$ Administration of formate and acetate did not result in labeling of oxalate Nakada and Weinhouse [35] studied glycine oxidation in rat liver and kidney homogenates and slices They showed that glycine 2 $C^{14}$ rapidly labeled carbon dioxide and hippurate but not oxalate Glycolate 2 $C^{14}$ or glyoxylate 1,2 $C^{14}$ rapidly labeled all three compounds and oxalate most extensively This suggested to them that glyoxylate was appreciably converted to oxalate only at high substrate concentrations When they incubated glycine-$C^{14}$ with pigeon liver homogenates in the presence of a large pool of unlabeled glyoxylate the glyoxylate became highly labeled and significant incorporation of $C^{14}$ into oxalate also resulted When glyoxylate was present in concentrations of 0 001 M or less it was oxidized to $CO_2$ and yielded little or no oxalate but as glyoxylate concentration was increased oxalate labeling was increased and at a glyoxylate concentration of 0 01 M oxalate was almost as highly labeled as $CO_2$ Since pigeon liver is devoid of xanthine oxidase activity these results confirm the existence of a second enzyme system capable of oxidizing glyoxylate to oxalate

The reactions by which oxalate may arise from glycine may be summarized as follows




GLYCOLIC ACID PATHWAY

Glyoxylic acid may be formed in mammalian tissue from precursors other than glycine via glycolic acid The chief sequence in which glycolic acid occurs is in the metabolism of ethanolamine formed by decarboxylation of serine [36–38]




Glyoxylic acid may be reduced to glycolic acid with lactic dehydrogenase and DPNH [35] The reverse reaction is catalyzed by a flavoprotein glycolic oxidase [36] and its operation is demonstrated by the conversion of glycolic acid to glyoxylic acid glycine and oxalic acid in the experiments of Nakada and Weinhouse discussed above

Large quantities of ingested oxalate may significantly depress calcium absorption from the gastrointestinal tract by forming the insoluble calcium oxalate salt [45–48] The effect of calcium intake upon oxalate absorption has not yet been studied

Archer et al [49] have presented data on the absorption of soluble and insoluble oxalates in 6 normal subjects On a diet with a mean oxalate content of 1 288 mg and a mean calcium content of 976 mg per 24 hr the urinary oxalate excretion ranged from 16 to 34 mg per 24 hr

Four subjects were then fed a solution of sodium oxalate in increasing quantities in four divided doses immediately after meals for six consecutive days The daily dose ranged from 1 0 to 4 0 gm expressed as oxalic acid dihydrate Urinary excretion of oxalate gradually increased over the 6 day period but the mean increases accounted for only 2 3 to 4 5 per cent of the administered dose Some subjects did not show an excretion of oxalate above the normal range until oxalate intake was increased at least 2 5 fold over the basal dietary level

*In an identical experiment 2 subjects were fed increasing doses of* calcium oxalate over a 6 day period The doses ranged from 1 3 to 4 3 gm as oxalic acid dihydrate The data show a slight increase in oxalate excretion but this is not significantly outside the normal range and not at all comparable to the increase observed during ingestion of soluble sodium oxalate

Dempsey studied 3 normal subjects on a metabolic ward and found that the ingestion of a diet rich in oxalate caused an increase in the 24 hr urinary excretion of oxalate two to four times that observed on a low oxalate diet [50] Instances of oxalate poisoning following rhubarb gluttony [1] or experimental feeding in animals have been recorded [51]

## OXALATE METABOLISM

Dakin suggested in 1922 that oxalate is an end product of metabolism and is probably not oxidized by mammalian tissues nor essential for any metabolic process [13] This has been substantiated by the finding that isotopically labeled oxalate given subcutaneously or intraperitoneally to rats is excreted unchanged in urine and feces although a small amount is stored in bone and muscle following large doses There is essentially no labeling of respiratory carbon dioxide or urinary hippurate [34 52 53] In human beings also labeled oxalic acid $C^{14}$ given orally or intravenously does not label expired carbon dioxide and intravenously administered oxalic acid is almost quantitatively recovered in the urine [54]

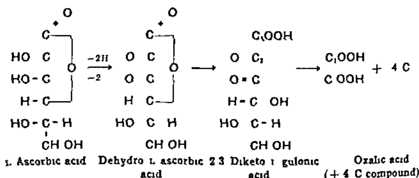
## OXALATE EXCRETION

The daily excretion of oxalate in urine has been determined by several techniques [49 55–59] it ranges from 9 0 to 50 0 mg as oxalic acid di

### ASCORBIC ACID PATHWAY

L-Ascorbic acid serves as a precursor of urinary oxalate in both animals and man but the specific mechanisms involved have not been elucidated Following intraperitoneal injection of labeled L-ascorbic acid in guinea pigs urinary oxalate was significantly labeled When $C^{14}$ was present only in the carboxyl group of the vitamin approximately 20 per cent of urinary $C^{14}$ appeared as oxalic acid in 48 hr, whereas a much smaller percentage was found in oxalic acid when uniformly labeled ascorbic acid was employed *[40]*

When 30 mg L-ascorbic acid 1 $C^{14}$ was given intravenously to three human subjects 10 per cent of the $C^{14}$ appeared in urine in 24 hr and 70 per cent in 50 days By far the largest quantity of urinary activity was present in oxalic acid which accounted for 44 per cent of the excreted label Hellman and Burns *[41, 42]* have suggested as a partial pathway, the following sequence



L Ascorbic acid Dehydro L ascorbic acid 2 3 Diketo L gulonic acid Oxalic acid (+ 4 C compound)

It is not known whether this pathway involves intermediary formation of glyoxylic acid

*Other Pathways*

Other pathways for the biosynthesis of oxalic acid have been found in microbial organisms and are discussed in a recent review by Jakoby and Bhat *[43]* The precursors identified are oxaloacetic acid oxalosuccinic acid and *β* ketoadipic acid

## OXALATE METABOLISM IN NORMAL MAN

### ABSORPTION

Until recently the major source of oxalate in the body was thought to be ingested foodstuffs particularly beets spinach rhubarb chocolate cocoa and tea The recent data of Andrews and Viser *[44]* may be consulted for the oxalate content of various foods

urinary oxalate may be derived from ascorbic acid However since only about 15 per cent of ascorbic acid carbon appears as oxalate carbon the average dietary intake of ascorbic acid would not account for more than a few milligrams of urinary oxalate each day

### THE MISCIBLE POOL OF OXALATE

The quantity of oxalate present in the body in rapidly miscible form has been determined in 3 normal subjects by isotope dilution techniques [63] Each subject was given 1 microcurie oxalic acid 1 2 $C^{14}$ intravenously following which the specific activity of urinary oxalate was determined in 2 to 4 hr specimens for 48 hr The miscible pool and the rate of its turnover were calculated from the linear portion of the specific activity decay curve

In these 3 subjects the miscible pool of oxalic acid ranged from 4 7 ± 0 7 to 8 6 ± 1 1 mg The turnover of the pool was rapid and half times of 2 2 to 2 8 hr were calculated The daily turnover of oxalate ranged from 34 0 ± 6 7 to 68 8 ± 11 3 mg in each case the turnover value approximated (and included within the range of M ± 2 s d ) the daily excretions of oxalate as determined by the method of Archer et al [49] From 89 to 97 per cent of the infused tracer was recovered unchanged in the urine in 36 hr

### OXALATE IN BODY FLUIDS

Many procedures have been devised to measure oxalate content of blood directly they have produced values ranging from 0 23 to 7 5 mg per 100 ml [65-70] Barrett evaluated the results obtained by several techniques and concluded that normal blood oxalate was approximately 0 5 mg per 100 ml but that no available method was highly specific or accurate [69] Verification will have to await a more sensitive accurate and specific method of determination

## METABOLIC STUDIES IN PRIMARY HYPEROXALURIA AND OXALOSIS

### SERUM OXALATE CONCENTRATION

Serum oxalate determinations have been reported in only one patient with oxalosis Marshall and Horwith [19] found values of 2 05 and 3 55 mg per 100 ml compared with a value of 1 45 mg per 100 ml on pooled normal serum

### OXALATE EXCRETION

The urinary excretion of oxalate in proved cases of oxalosis has ranged from 53 to 370 mg per day (Table 14 1) Oxalate to creatinine ratios have ranged up to 0 193 mg $(COOH)_2 \cdot 2H_2O$ per mg creatinine [normal—0 02 mg $(COOH)_2 \cdot 2H_2O$ per mg creatinine] [11] In one patient oxalate

hydrate By the method of Archer et al [49] the normal urinary oxalate excretion ranges from 9 0 to 45 0 mg per day on an average diet

In three subjects no consistent diurnal variation was observed between successive 8 hr specimens and excretion of oxalate was not significantly correlated with urine volume [54]

In normal subjects the urinary excretion of oxalate was not affected by ingestion of glycine in quantities ranging from 25 gm as a single dose [54] to 100 gm in divided doses daily for 6 days [12] In 3 subjects the 24 hr excretion of oxalate was doubled following ingestion of 40 gm gelatin which contains 25 per cent glycine [50] Attempts to reduce oxalate excretion by the daily ingestion of 4 to 40 gm sodium benzoate were unsuccessful in 2 subjects [12] Administration of 10 to 20 mg pyridoxine hydrochloride to mentally defective children resulted in a 25 to 58 per cent decrease in the 8 hr overnight excretion of oxalate [60] In 3 normal adult males ingestion of 20 mg pyridoxine hydrochloride was followed by a mean reduction of 29 3 per cent in overnight excretion of oxalate [54] These reductions may represent an effect on transamination reactions for which pyridoxal 5 phosphate serves as a cofactor [61] *diverting glycine or serine or both from conversion to oxalate* After massive dose of ascorbic acid urinary oxalate excretion may be increased In two studies ingestion of 9 gm ascorbic acid daily for 2 days led to increases in mean oxalate excretion from 41 to 109 mg per day [62] and from 26 to 40 mg per day [54] in 11 and 3 normal subjects respectively

The mechanism by which the kidneys excrete oxalate is unknown

## BIOSYNTHESIS OF OXALATE IN MAN

Following oral administration of glycine 1 $C^{14}$ or 1 $C^{13}$ [63, 63a] to normal subjects urinary oxalate is highly labeled within a few hours When glycine 1 $C^{13}$ was fed repetitively to 1 control subject until urinary products achieved constancy of isotopic enrichment oxalate was approximately 40 per cent as highly labeled as free glycine isolated from the same samples [63a] These results establish glycine as a precursor of perhaps 40 per cent of the urinary oxalate in this subject and suggest that the biosynthetic pathway is relatively direct and involves little dilution in pools of intermediates The most direct pathway would be glycine ⟶ glyoxylic acid ⟶ oxalic acid

In 5 controls an average of 0 051 per cent (range 0 029 to 0 081 per cent) of a single oral dose of glycine 1 $C^{14}$ appeared in urinary oxalate Since their mean urinary excretion of oxalate (35 mg per day) approximates 0 035 to 0 07 per cent of the daily turnover of the glycine pool (0 5 to 1 0 gm per kg [64]) the incorporation values cited above suggest that a large portion of urinary oxalate may have been derived from glycine in these subjects

Hellman and Burns [41] have postulated that the major portion of

excretion declined terminally to low levels (3 to 20 mg per day) but the urinary oxalate to creatinine ratio remained high [0 08 to 0 10 mg $(COOH)_2 2H_2O$ per mg creatinine] [*13*]

Archer et al [*12*] have conducted a series of metabolic studies on 2 patients with primary hyperoxaluria and oxalosis in which they evaluated the effect on urinary oxalate excretion of the inge tion of oxalates and of measures de igned indirectly to alter body glycine stores

*Oxalate Ingestion*

Following inge tion of odium oxalate and of rhubarb juice [containing about 4 gm of $(COOH)_2 2H_2O$ per liter] both hyperoxaluric subjects showed small increa es in urinary oxalate The increments corre ponded to 1 2 to 5 4 per cent of the ingested quantities and thus agreed clo ely with values obtained earlier following ingestion of soluble oxalate in normal subjects Calcium oxalate ingestion did not materially alter the level of urinary oxalate

*Dietary Protein Restrictions*

One of two patients showed a mall but statistically significant de crease in urinary oxalate excretion during protein re triction whereas the second patient showed no significant change de pite a greater re striction of dietary protein

*Sodium Ben oate Administration*

Sodium benzoate administration (10 to 40 gm per day) was associated with a transient decrease in urinary oxalate excretion in both hyper oxaluric patients In the first the mean urinary oxalate excretion de creased approximately from 200 to 165 mg per day in the second ap proximately from 95 to 65 mg per day In the latter patient a restricted protein intake did not enhance the response to sodium benzoate

Prolonged sodium benzoate administration (20 gm per day) was un successful in achieving a permanent reduction in oxalate excretion in one patient After about 4 weeks the excretion of oxalate gradually returned to high levels

*Glycine Administration*

In one patient the administration of 18 gm glycine per day for 8 days was as ociated with a slight rise in average oxalate excretion approxi mately from 135 to 165 mg per day Marshall and Horwith [*19*] report that oxalate excretion was increa ed following oral ingestion of glycine glycolic acid and ascorbic acid in their subject

## TRACER STUDIES WITH LABELED GLYCINE

Scowen Crawhall and Watts [*71 71a*] administered glycine 1 $C^{14}$ orally every 6 hr for 4 days to patients with primary hyperoxaluria and

TABLE 14 1 SUMMARY OF PROVED CASES OF PRIMARY HYPEROXALURIA AND OXALOSIS

| Auth | R fer | Y r | S | Ag at onset of sympt m y | Age d th yr | Urinary oxal te exc t mg (COOH) H O/ 4 hr | Renal calc d po t | Ext al rilat d po its |
|---|---|---|---|---|---|---|---|---|
| Dav a 1 1 gbe g nd St ll | [3] | 1950 | [illegible] | 3 | 12 | | + | + |
| Ch l D l | [4] | 1952 | M | 1 1/2 | [illegible] | | + | + |
| Zoll ger nd R m nd | [ ] | 195? | M | 3 1/2 | 4 1/2 | | + | + |
| N w d Bl k | [ ] | 19 3 | F | 2 | 8 1/2 | 95–182 | No aut ) | No autopsy |
| Ar t d F tt | [6] | 1954 | M | 5 | 16 | 00 | + | + |
| Ar t nd F tt | [6] | 1954 | M | 10 | 13 1/2 | 180 | + | + |
| B k et al | [ ] | 1955 | M | 3 | 11 1/2 | | + | + |
| D n | [9] | 1955 | F | 4 | 15 | | + | + |
| N t St n d K ne | [10] | 1955 | M | 1 1/2 | 5 | | + | + |
| L nd— t d by A l et l [11] | | 1957 | M | 3 | ? | | + | + |
| A l et l | [11 13] | 19 7 | M | 1 | 3 | 1[illegible] 3 0 | + | + |
| A l et l | [11 13] | 1957 | F | 4 | 10 ( 1 ) | 110– 65 | + | ? |
| S mk | [14] | 1957 | F | 1 1/2 | 4 1/2 | | + | + |
| H ll | [15] | 1957 | | | | | | |
| G l F t D mpsey nd H n m n | [17] | 19 8 | M | 5 | 10 | 130–180 | + | + |
| Wy g rd n V r nd Ell | [18 7J] | 1958 19(0 | M | 11 | 31 ( l e) | 103 143 | ? | ? |
| K t un and Sa dbank | [18] | 19 9 | F | 1/2 | 4 1/2 | | + | + |
| M rsh ll nd H tt | [ ] | 19 9 | F | 6 | 18 | | + | + |
| M sh ll d H w th | [19] | 19 9 | F | 17 | 4 | | + | + |
| M sh ll l H tt | [19] | 1959 | F | 1 1/2 | 0 ( 1 ) | 53 71 | + | + |

in whom neither hyperoxaluria nor extrarenal deposits of calcium oxalate have been demonstrated Patients in this category have been described in age ranging from 5 months to 2 years [72–76] (2) Patients with calcium oxalate nephrolithiasis or nephrocalcinosis or both in whom extrarenal deposits of calcium oxalate have been demonstrated at autopsy but in whom the disorder had its onset in middle adult life or occurred concomitantly with and perhaps as a consequence of another serious renal disease [16, 77 78] (3) Patients with calcium oxalate nephrolithiasis and hyperoxaluria but without nephrocalcinosis or known tissue deposits of oxalate in whom symptomatic disease first began in adult life [50 79]

A number of patients of the first two groups have been excluded from Table 14 1 because of incomplete pathologic studies of tissues or because of onset of the disease in adult life Further study may disclose that the adult cases are indeed authentic examples of the primary disorder, but until more is known about conditions leading to secondary hyperoxaluria they had best be placed in an uncommitted category Patients of the third group are discussed more fully below in connection with the general problem of oxalate nephrolithiasis

## OXALATE NEPHROLITHIASIS

### INCIDENCE AND ETIOLOGIC FACTORS

In the United States from 22 to 70 per cent of all urinary tract stones are composed of oxalate [80–86] This wide discrepancy reflects different criteria for classification In the so called stone belt of central Europe the frequency of oxalate stones is estimated as high as 65 per cent of all urinary tract calculi [87]

The etiology of oxalate nephrolithiasis is poorly understood Oxalate calculi may form in acid neutral or alkaline urine and remain insoluble in either acid or alkaline urine Neither infection nor stasis is so important a predisposing factor in oxalate nephrolithiasis as in calcium phosphate nephrolithiasis [1 31 86]

In a number of areas of the world where oxalate stones are particularly prevalent a high oxalic acid content of the diet has been implicated as an etiologic factor In other areas where oxalate stones are common the diet is extremely low in oxalic acid content [31 87–89]

Normal urine is usually supersaturated with calcium oxalate up to six or eightfold according to Joly [27 31] The increased solubility of calcium oxalate in urine over that in water has been attributed to the presence of urea and colloidal substances [29 90] It has been suggested that oxalate nephrolithiasis results from decreased solubility of calcium oxalate in urine because of a disturbance involving such a protective colloidal mechanism but this remains to be demonstrated At times hypercalcinuria is a contributing factor [50] In most patients it is

found a prompt and considerable incorporation of the isotope into urinary oxalate When constancy of isotopic enrichment had been achieved urinary oxalate was 50 and 32 per cent as highly labeled as free urinary glycine in the 2 subjects in whom glycine enrichment was measured Thus approximately the same fraction of urinary oxalate appeared to be derived from glycine in these 2 hyperoxaluric subjects as in 1 control subject

In one hyperoxaluric subject, Wyngaarden Verner and Elder [63, 79] found incorporation of 0 22 per cent of a single oral tracer dose of glycine 1 $C^{14}$ into urinary oxalate This value is some fourfold greater than the mean normal value This patient excreted 103 to 143 mg oxalic acid dihydrate per day, an amount about fivefold greater than the mean normal excretion value Thus these results also suggest that about the same per centage of urinary oxalate is derived from glycine in this subject as in normal subjects

Even in the most marked cases of hyperoxaluria the urinary excretion of oxalate amounts only to approximately 0 5 per cent of the total daily glycine turnover [64] No studies have been performed with labeled glyoxylate or ascorbic acid in hyperoxaluric subjects

## THE METABOLIC DEFECT IN PRIMARY HYPEROXALURIA

The data presented above indicate that the large amount of oxalate present in the urine and tissues of patients with oxalosis is of endogenous origin They show that glycine is a precursor of about the same per centage of the urinary oxalate in patients with primary hyperoxaluria as in control subjects The basic abnormality in primary hyperoxaluria is therefore probably a failure of one or another of the reactions by which glyoxylate is metabolized other than to oxalate viz transamination to glycine reduction of glycolic acid or oxidative degradation to formate and carbon dioxide rather than excessive endogenous production of glyoxylate from glycine

Whatever the defect it appears to be severe and lifelong No cofactors are known that are unique to glyoxylate metabolism and no metabolic defect is known to exist in hyperoxaluric subjects other than overproduction of oxalate It is reasonable therefore to postulate that an enzymatic step concerned directly or indirectly with glyoxylate metabolism may be defective in this disorder

### RELATED CASES OF UNCERTAIN CLASSIFICATION

There are three groups of patients in whom certain features suggest the diagnosis of primary hyperoxaluria and oxalosis but in whom the actual relationship if any to the authentic syndrome is at present unclear These are (1) Patients with nephrocalcinosis due to calcium oxalate

[63] Prior to dietary protein restriction the miscible pool of oxalate was 39 1 mg and its turnover 110 mg per day following decline of oxalate excretion the miscible pool was 1 8 mg and its turnover 8 2 mg per day Thus the decline in excretion was secondary to a remarkable decrease in the content of soluble oxalate within body fluids The great increase in turnover (and excretion) demonstrated on initial studies could have been secondary to increased production or increased absorption of oxalate Subsequently an oxalate absorption study was conducted in the fasting state at a time when urinary oxalate was low [54] Forty four per cent of a 50 μg dose of oxalic acid 1 2 $C^{14}$ was absorbed and appeared in urine This was somewhat greater than was found under similar conditions in 2 control subjects (one hypercalcinuric) who excreted 24 and 35 per cent of the oral dose as urinary oxalate

## EXPERIMENTAL OXALATE NEPHROLITHIASIS OXALATE NEPHROCALCINOSIS AND HYPEROXALURIA

Keyser [94 94] produced oxalate calculi in dogs and rabbits following subcutaneous administration of oxalic acid esters and calcium salts Rost produced renal calculi in dogs by feeding sodium oxalate [94a] Hammarsten [95 96] produced oxalate stones in rats on a magnesium poor diet A deficiency in vitamins A and D as well as additional oxalates in the diet favored stone formation but was not e sent il since oxalate stones also formed in magnesium-deficient rats on an oxalate-free diet The magnesium deficiency per se was not specific for oxalate calculus formation These stones predominated when urine pH was acid whereas calcium phosphate stones formed when it was alkaline

Gershoff and associates [97] produced an experimental vitamin $B_6$ deficiency in the cat characterized by failure of growth emaciation convul ions anemia and oxalate nephrocalcinosis Exces ive endogenous formation of oxalate led to hyperoxaluria but gross calculi were not seen at any level of the urinary tract Extrarenal deposits of calcium oxalate were not detected but the experiments were of relatively short duration Extensive glomerular lesions and deep cortical scars unlike the renal lesions of human oxalo is were observed in these cats By feeding oxalic acid and oxamide Ebstein and Nicolaier [98] produced renal lesions in dogs which were comparable to those formed in the vitamin $B_6$-deficient cats

## GENETICS

Archer and associates [99] have made detailed studies of urinary oxalate excretion among relatives of three patients proved to have primary hyperoxaluria No abnormally high level of urinary oxalate was en countered among the siblings parents first cousins uncles and aunts and more distant relatives Among decea ed relatives a history ug

probable that transient dehydration and formation of very concentrated urine are important predisposing factors in oxalate stone formation

## OXALATE EXCRETION

Urinary oxalate excretion is normal in most patients who form oxalate stones [*50 91, 92*], factors other than hyperoxaluria appear to be responsible Nevertheless as pointed out above there exists a group of patients who form calcium oxalate stones with varying frequency, in whom hyperoxaluria of mild or even severe degree has been found The relationship to the syndrome of primary hyperoxaluria is obscure

In 1957, Dempsey [*50*] reported studies of oxalate and calcium excretion in a group of 45 patients with calcium oxalate stones in selected patients with idiopathic or secondary hypercalcinuria and in patients with hepatic cirrhosis Ten subjects had hyperoxaluria (50 to 110 mg per day) including 5 of 6 patients with hepatic cirrhosis (70 to 80 mg per day) In this study all the hyperoxaluric subjects who formed calcium oxalate stones also had hypercalcinuria but a number of the hypercalcinuric patients had normal values for urinary oxalate In one young patient who had primary hyperoxaluria and who was subsequently shown by Godwin et al [*17*] to have oxalosis urinary oxalate excretion ranged from 130 to 180 mg per day and the urinary calcium excretion was normal as in other patients with primary hyperoxaluria

A study [*54 79*] of 12 selected patients with a long history of recurrent nephrolithiasis due to calcium oxalate stones may also be cited In this group 4 patients excreted normal quantities of oxalate in urine [12 to 45 mg per day as $(COOH)_2 \cdot 2H_2O$] 3 excreted between 50 and 100 mg per day and 5 excreted from 103 to 238 mg per day In 2 patients of the latter group values of urinary oxalate eventually declined to normal In 1 in whom no dietary changes were made values declined from 110 to 41 mg per day during approximately 4 months of observation In the other (who also showed hypercalcinuria) dietary restriction of oxalate protein and calcium intake was accompanied by a prompt decline in urinary oxalate from 117 to 11 mg per day Concomitantly this patient's urine became free of crystals of calcium oxalate for the first time in several years

## METABOLIC STUDIES

Studies of incorporation of glycine 1 $C^{14}$ into urinary oxalate were performed in the adult hyperoxaluric hypercalcinuric subject [*79*] The test was done during dietary restriction when urinary oxalate had declined to 54 mg per day The patient incorporated 0 047 per cent a normal value Additional studies are necessary to determine the origin of the urinary oxalate in this subject

A limited number of additional observations was made on this patient

and biochemically by a continuous high urinary oxalate excretion Extra renal deposits of calcium oxalate occur as a stage in the natural history of the disea e Their presence establi hes the additional diagno is of oxalosis The disorder usually begins in early childhood and leads to death from renal failure in childhood or early adult life No effective therapy is known

2 The hyperoxaluria is due to a metabolic defect leading to endogenous overproduction of oxalate About the same percentage of urinary oxalate arises from glycine in hyperoxaluric subjects as in normal subjects On theoretical grounds a defect in metaboli m of glyoxylic acid the only *known direct precursor of oxalic acid is suspected but a preci e bio* chemical localization of the metabolic defect is not yet po ible

3 The di order is thought to be genetically determined and pre liminary evidence suggests that it may be inherited as an auto omal rece ive trait No increase in urinary excretion of oxalate has been found in individuals who are presumed to be heterozygous for the trait

## BIBLIOGRAPHY


1 Jeghers H and Murphy R Practical aspects of oxalate metabolism New England J Med **233** 08 938 1945
2 Lepoutre Calculs multiples chez un enfant infiltrat on du parenchyme rénal par des dépôts cristallins J urol Paris **20** 4 4 1) 5
3 Davis J S Klingberg W G and Stowell R E Nephrol thiasis an l nephro calcino is with calcium oxalate crystals in kidneys and bones J Pediat **36** 3 3 1950
4 Chou L Y and Donohue W L Oxalosis Po sible inborn error of metabolism with nephrol thiasis and nephrocalcino i due to cal ium oxalat as the pre dominating features *Ped at ics* **10** 6C0 1952
5 Newn C H and Black J A Calcium oxalate nephrocalcinosi Great Ormond St J **5** 40 1953
6 Aponte G E and Fetter T R Familial idiopathic oxalate nephrocalcinosis Am J Clin Path **24** 1363 1954
7 Zollinger H V and Rosenmund H Uramie bei endogen bedingter subakuter und chronischer Calcium oxalatn ere (Calciumoxalatt epl ritis und Calciumoxalat schrumpfniere) Schweiz med Wchnschr **82** 1261 1J5
8 Burke L C Baggenstoss A H O en C A Jr Po er M H and Lohr O W Oxalosis Pediatrics **15** 383 1955
9 Dunn H G Oxalo is A M A Am J D Child **90** 58 1955
10 Neu tein H R Stevenson S S and Kramer I Oxalo is with r nal calcinosis due to calc um oxalate J Pe liat **47** 624 1955
11 Archer H E Dormer A E Scowen E F and Watt R W L Primary hyperoxaluria Lancet **2** 3 0 1955
12 Archer H E Dormer A E Scowen E F and Watts R W E The aetiology of primary hyperoxaluria Lancet **1** 1 5 1958
13 Scowen E F Stansfield A G and Watts R W E Oxalo and p mary hyperoxaluria J Path & Bact **77** 1 5 1959
14 Smko I Oxalo is Ann paediat **189** 1 1955
15 Hollo i K Oxalo e Zentr bl allg l ath **96** 20 195
16 Edward D I Hopathic familial oxal is A M A Arch I th **64** 546 1957
17 Colvin J T Fowler M E Dempsey L F and Henneman I H Primary hypero alu ia and oxalo is Report of a case and review of the l terature New Engl nd J Med **259** 1099 1958

gestive of primary hyperoxaluria was obtained in only one sibling of one of the propositi There was no history of consanguinity in any of the three families

Archer et al have made several cogent preliminary generalizations (1) They point out that no relevant abnormality has been established in the parents of a single case of primary hyperoxaluria except for the father of Dunn s [9] case who had passed a urinary calculus Urinary oxalate determinations were not recorded in Dunn s study (2) No cases arising from consanguineous parents have yet been reported (3) Males predominate but females with this disorder are afflicted with an equally severe disease (4) Most of the cases have occurred as isolated examples in a family but both Newns and Black [5] and Aponte and Fetter [6] have reported affected siblings including identical twins in the latter family Archer et al suggest that these data are compatible with the hypothesis that primary hyperoxaluria is due to the operation of a rare recessive character

Since the urinary excretion of oxalate was normal in all family members studied by Archer et al [99] the detection of individuals heterozygous for the defective gene is presently impossible Perhaps a suitable stress such as oral loading doses of glycine or glyoxylate would lead to hyperoxaluria and permit their detection One wonders whether any of the adult subjects with recurrent oxalate stones and possibly transient hyperoxaluria may represent such carriers of the gene for primary hyperoxaluria responding to an unknown provocative stress with an increase in endogenous production of oxalate

The possibility that genetic factors may influence stone formation without operating through mechanisms concerned with oxalate production cannot be excluded on the basis of available evidence In 1931 Gram [100] published an extensive pedigree of five generations of a family in which a number of cases of calcium oxalate urolithiasis had arisen Urinary oxalate determinations were not reported and the clinical courses did not suggest that they were examples of primary hyperoxaluria

## TREATMENT IN PRIMARY HYPEROXALURIA

No successful treatment directed at the fundamental clinical problem persistent hyperoxaluria is available Measures shown to reduce excretion of oxalate in certain other patients with hyperoxaluria viz protein restriction and administration of vitamin $B_6$ and of sodium benzoate warrant further trial

## SUMMARY

1 Primary hyperoxaluria is a rare disorder characterized clinically by progressive bilateral calcium oxalate urolithiasis and nephrocalcinosis

47 Fincke M L and Sherman H C Availability of calcium from some foods J Biol Chem 110 421 1935
48 Adolph W H Production of rickets in rats fed diets containing o Chinese J Physiol 14 51 1939
49 Archer H E Dormer A E Scowen E F and Watts R W E Studies on the urinary excretion of oxalate by normal subjects Clin Sc 16 405 1957
50 Dempsey E F Urinary oxalate excretion in relation to renal stone formation J Clin Invest 36 88? 1957
51 Dakin H D *Oxidations and Reductions in the Animal Body* 2d ed Monographs on Biochemistry London 19?
52 Brubacher G Just M Bodur H and Bernhard K Zur Biochemie der Oxalsaure Ztschr physiol Chem 304 173 1956
53 Curtin C O H and King C G The metabolism of ascorbic acid 1 C[1] and oxalic acid C in the rat J Biol Chem 216 539 1955
54 Elder T D and Wyngaarden J B Unpublished data
55 Herkel W and Koch K Untersuchungen zur Oxalsaure Ausscheidung insbesondere bei Nierensteinkranken Deutsches Arch klin Med 178 511 1936
56 Muller P Zur Biologie der Oxalsäure Physiol Abstr 22 349 1937 Verhandl schweiz Ver Physiol u Pharmakol 11 8 1937
57 Widmark E M P Studies on oxalic acid amount in urine Acta med scandinav Suppl 26 340 1928
58 Barrett J F Absorption and excretion of oxalate Lancet 2 5 4 1942
59 Powers H H and Levatin P A method for the determination of oxalic acid in urine J Biol Chem 154 207 1944
60 Gershoff S N Mayer S B and Kulczycki L L Effect of pyridoxine administration on the urinary excretion of oxalic acid pyridoxine and related compounds in Mongoloids and non Mongoloids Am J Clin Nutrition 7 76 1959
61 Snell E E Chemical structure in relation to biological activities of vitamin B Vitamins & Hormones 16 77 1958
62 Lamden M P and Chrystowski G A Urinary oxalate excretion by man following ascorbic acid ingestion Proc Soc Exper Biol & Med 85 190 1954
63 Elder T D and Wyngaarden J B The synthesis and turnover of oxalic acid in normal and hyperoxaluric subjects J Clin Invest 38 1001 1959
63a Crawhall J C de Mowbray R R Scowen E F and Watts R W E Conversion of glycine to oxalate in a normal subject Lancet 2 810 1959
64 Watts R W E and Crawhall J C The first glycine metabolic pool in man Biochem J 73 277 1959
65 Suzuke S Jap J M Sc II Biochem 2 291 1934 Physiol Abstr 19 675 1934
66 Steinberg A and Brown W R New concept regarding mechanism of clotting and control of hemorrhage Am J Physiol 126 638 1939
67 Steinberg A Segal H I and Parris H M Role of oxalic acid and certain related dicarboxylic acids in treatment and control of hemorrhage Ann Otol Rhin & Laryng 49 1008 1940
68 Barber H H and Gallimore E J The metabolism of oxalic acid in the animal body Biochem J 34 144 1940
69 Barrett J F B The oxalate content of blood Biochem J 37 254 1943
70 Dodds E C and Gallimore E J Determination of small quantities of oxalic acid Biochem J 26 1?4 193
71 Scowen E F Crawhall J C and Watts R W E Incorporation of carboxyl carbon atom of glycine into oxalate in a case of primary hyperoxaluria Lancet 2 300 1958
71a Crawhall J C Scowen E F and Watts R W E Conversion of glycine to oxalate in primary hyperoxaluria Lancet 2 806 1959
72 Lax L Oxalatablagerungen und Schrumpfnierenkrankheit in den Nieren Frankfurt Ztschr Path 55 26? 1941
73 Walter W Calciumoxalatschrumpfniere mit Uraemie Schweiz Ztschr allg Path 10 286 1947

18 Katzum E and Sandbank U Oxalosis Arch Dis Childhood 34 60 1959
19 Marshall V F and Horwith M Oxalosis J Urol 82 278 1959
20 Cogan D G Kuwabara T Silbert J Kern H McMurray V and Hurlburt C Calcium oxalate crystals in detached retinas A M A Arch Ophth, 60 366 1958
21 Zimmerman L E and Johnson F B Calcium oxalate crystals within ocular tissues A M A Arch Ophth 60 372 1958
22 Albright F Sulkowitch H W and Chute R Non-surgical aspects of the kidney stone problem J A M A 113 2049 1939
22a Caffey J *Pediatric X ray Diagnosis* p 960 Year Book Publishers Inc Chicago 1956
23 Johnson F *A method for demonstrating calcium oxalate in tissue sections* J Histochem & Cytochem 4 404 1956
24 Kobert R and Kussner F Die experimentellen Wirkungen der Oxalsaure Arch path Anat 78 209 1879
25 Ebstein W and Nicolaier A Uber die Wirkung der Oxalsaure und einige ihrer Derivate auf die Nieren Arch path Anat 148 366 1897
26 Dunn J S Haworth A and Jones N A The pathology of oxalate nephritis J Path & Bact 27 299 1924
27 *Joly J S Some points in etiology of primary urinary calculi Urol & Cutan* Rev 43 637 1939
28 Lassen H K Formation of urinary calculi J Urol 60 110 1943
29 Medes G Solubility of calcium oxalate and uric acid in solutions of urea Proc Soc Exper Biol & Med 30 281 1932
30 Maclagan N F and Anderson A J Some observations on urinary colloids in relation to renal calculi Brit J Urol 30 269 1958
31 Joly J S *Stone and Calculous Disease of the Urinary Organs* Mosby St Louis 1940
32 Ratner S Nocito V and Green D E Glycine oxidase J Biol Chem 152 119 1944
33 Cammarata P S and Cohen P P The scope of the transamination reaction in animal tissues J Biol Chem 187 439 1950
34 Weinhouse S and Friedmann B Metabolism of labeled 2-carbon acids in the intact rat J Biol Chem 191 707 1951
35 Nakada H I and Weinhouse S Studies of glycine oxidation in rat tissues Arch Biochem 42 257 1953
36 Kun E Dechary J M and Pitot H C The oxidation of glycolic acid by a *liver enzyme J Biol Chem 210 269 1954*
37 Elwyn D Weissbach A Henry S S and Sprinson D B The biosynthesis of choline from serine and related compounds J Biol Chem 213 281 1955
38 Narrod S A and Jakoby W B Enzymatic conversion of ethanolamine to glycolaldehyde Fed Proc 17 281 1958
39 Nakada H I and Sund L P Glyoxylic acid oxidation by rat liver J Biol Chem 233 8 1958
40 Burns J J Dayton P G and Schulenberg S Further observations on the metabolism of L-ascorbic acid in guinea pigs J Biol Chem 218 15 1956
41 Hellman L and Burns J J Metabolism of L-ascorbic acid 1 C in man J Biol Chem 230 923 1958
42 Burns J J Biosynthesis of L-ascorbic acid basic defect in scurvy Am J Med 26 740 1959
43 Jakoby W B and Bhat J V Microbial metabolism of oxalic acid Bact Rev 22 75 1958
44 Andrews J C and Viser E T The oxalic acid content of some common foods Food Res 16 306 1951
45 Fairbanks R W and Mitchell H H Availability of calcium in spinach in skim milk powder and in calcium oxalate J Nutrition 16 79 1938
46 Tisdall F F and Drake T G H Utilization of calcium J Nutrition 16 613 1938

Chapter 15

# Maple Syrup Urine Disease*

*Joseph Dancis and Mortimer Levitz*

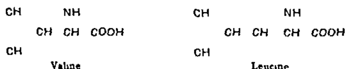
## HISTORICAL NOTE

In 1954 Menkes Hurst and Craig described a family in which four out of six infants one girl and three boys died during the first weeks of life with what appeared to be a congenital metabolic disease [1] The prominent signs were vomiting muscular hypertonicity and a maple syrup odor to the urine Examination of the urine by paper chromatography failed to reveal an abnormality in the amino acid pattern and no clue could be found to the metabolic anomaly

In 1957 Westall Dancis and Miller described a 20 month-old infant (Boy A) who was mentally retarded had muscular hypertonicity and a maple syrup odor to the urine [2] A detailed account has appeared more recently [3] An excess of leucine was found in the urine of this patient on analysis by paper chromatography Further investigation (4) suggested a metabolic block in the degradative pathway of the branched chain amino acids An additional finding was an elevated methionine and low cystine level in the plasma Mackenzie and Woolf [5] have reported a second case (Girl B) with the same metabolic anomaly

## METABOLISM OF THE BRANCHED CHAIN AMINO ACIDS

Valine leucine and isoleucine are referred to as the branched chain

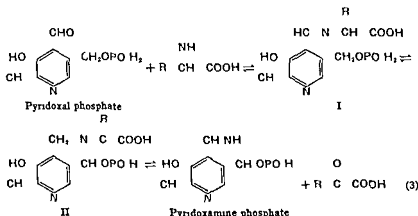



This work was supported in part by the Association for Aid to Crippled Children and a grant (40 1) from the Public Health Service

74 Mulloy M Knutti R E and Coettsch E An unusual case of calcium oxalate deposits in the kidneys of a young infant J Pediat 39 ol 1951
75 Ostry H Nephrocalcinosis Can M A J 65 465 1951
75a Orgaard H and Soderhjelm L Familial oxalosis Acta soc med upsalien 62 176 1957
76 Laas I Oxalatablagerungen und Sammelrohrennekrosen in den Nieren Frankfurt Ztschr Path 55 265 1941
77 Arons W L Christensen W R and Sosman M C Nephrocalcinosis visible by x ray associated with chronic glomerulonephritis Ann Int Med 24 260 1955
78 Lund T and Reske Nielsen F Nephrolithiasis and nephrocalcinosis with calcium oxalate crystals in the kidneys and other organs Acta path et microbiol scandinav 38 353 1956
79 Wyngaarden J B and Verner J V The biosynthesis of oxalate from glycine 1 C14 in man Clin Res 6 267 1958
80 McIntosh J F Classification and chemical pathogenesis of urinary calculi J Clin Invest 21 755 1942
81 Kretschmer H L Stone in ureter clinical data based on 500 cases Surg Gynec & Obst 74 1065 1942
82 Randall A Origin and growth of renal calculi Ann Surg 105 1009 1937
83 LaTowsky L W Qualitative chemical analysis of urinary calculi J Urol 49 720 1943
84 Barney J D and Jones G E Some problems in management of urinary calculi J Urol 45 1 1941
85 Burkland C E Etiology and prevention of oxalate calculi in urinary tract plan of therapy J Urol 46 8 1941
86 Thompson H E Steadman L T Benjamin J A and Scott W W Quantitative microchemical and spectrographic data on renal calculi and their relation to infection J Urol 51 259 1944
87 Grossman W Current urinary stone wave in central Europe Brit J Urol 10 46 1938
88 McGarrison R Causation of stone in India Brit M J 1 1009 1931
89 Siddell A C Primary vesical calculus J Urol 37 268 1937
90 Snapper I Bendein W M and Polak A Observations on formation and prevention of calculi Brit J Urol 8 337 1936
91 Melick R A and Henneman P H Clinical and laboratory studies of 207 consecutive patients in a kidney stone clinic New England J Med 259 307 1958
92 McIntosh J F and Read M K Oxalic acid excretion in oxalate lithiasis J Urol 80 272 1958
93 Keyser L D Mechanism of formation of urinary calculi Ann Surg 77 210 1923
94 Keyser L D Etiology of urinary lithiasis Arch Surg 6 525 1923
94a Rost I Toxicology of oxalic acid and oxalates J Pharmacol & Exper Therap 29 25 1926
95 Hammarsten G Eine experimentelle Studie uber Calciumoxalat als Steinbildner in den Harnwegen Lunds Univ Års kr 32 1935 1936
96 Hammarsten G Dietetic therapy in formation of calcium oxalate calculi in urinary passages Skand Arch Physiol 80 165 1938
97 Gershoff S N Faragalla F F Nelson D A and Andrews S B Vitamin B6 deficiency and oxalate nephrocalcinosis in the cat Am J Med 27 72 1959
98 Ebstein W and Nicolaier A Ueber die Wirkung der Oxalsaure und einiger ihrer Derivate auf die Nieren Arch path Anat 148 366 1897
99 Archer H E Dormer A E Scowen E F and Watts R W E Observations on the possible genetic basis of primary hyperoxaluria Ann Human Genet 22 373 1958
100 Gram H C The heredity of oxalic urinary calculi Acta med scandinav 78 268 1932

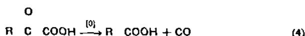
pecific [10] Even the more specific transaminases will occasionally retain activity when the receptor or donor is replaced by a substrate of similar structure [9]

The cofactor which mediates transamination is vitamin $B_6$ in either the pyridoxal or pyridoxamine form [11] *A plausible mechanism which* explains the coenzymatic nature of the vitamin is shown in Reaction 3 [11 12] A reversible enzymatic reaction between pyridoxal phosphate and the amino acid on the enzyme surface produces the Schiff base (I)

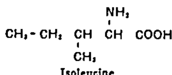



Simultaneous hydrogen and double bond shifts result in the isomeric Schiff base (II) which then splits into the keto acid and pyridoxamine phosphate The reversibility of the reaction permits the transfer of the amino group from pyridoxamine phosphate to the keto acid acceptor which is usually α ketoglutarate This completes the transamination reaction

The α keto acids are degraded further by irreversible oxidative decarboxylation (Reaction 4)



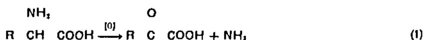

This reaction has been well documented for pyruvic and α ketoglutaric acids Direct evidence for the participation of the branched chain keto acids is lacking probably because the decarboxylated acid is rapidly metabolized to products of greater interest to investigators Kinnory Takedo and Greenberg [13] demonstrated the conversion of valine to α ketoisovaleric and isobutyric acid by rat liver homogenate Presumably degradation occurs through the keto acid to isobutyric acid Bloch [14] found that the rat degrades leucine and isovaleric acid to acetate in a similar fashion and suggested that leucine is converted to

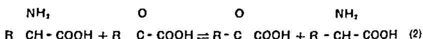


Isoleucine

amino acids because each contains a methyl group which is not part of the longest carbon to carbon sequence In common with the other amino acids the branched chain amino acids are either used in protein or polypeptide synthesis or are degraded extensively

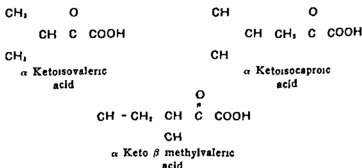
The catabolism of valine leucine and isoleucine is initiated by the loss of the amino group either by oxidative deamination (Reaction 1) or by transamination (Reaction 2) In oxidative deamination the enzyme L-amino acid oxidase liberates ammonia with the formation of the α keto acid The branched chain amino acids are relatively good substrates for


$$R\ CH(NH_2)\ COOH \xrightarrow{[O]} R\ C(O)\ COOH + NH_3 \quad (1)$$


$$R\ CH(NH_2)-COOH + R\ C(O)-COOH \rightleftharpoons R-C(O)\ COOH + R-CH(NH_2)-COOH \quad (2)$$

L-amino acid oxidase but the low enzymatic activity and the limited distribution of the enzyme make this pathway insignificant [6]

On the other hand transamination occurs in most animal tissues, and virtually all the natural amino acids participate [7] Transamination differs from oxidative deamination in that free ammonia is not involved [8] Alpha ketoglutaric acid is the most common of several α keto acids which may function as an $—NH_2$ receptor in enzymatic transamination It couples effectively with valine leucine and isoleucine converting them to the respective α keto acids α ketoisovaleric acid α ketoisocaproic acid and α keto β methylvaleric acid [9]

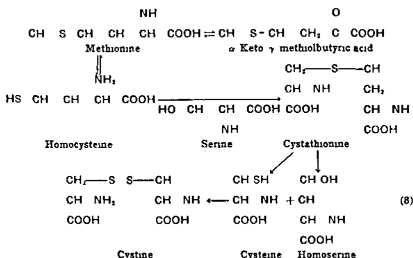



The literature on the specificity of the transaminases is too extensive and at times too contradictory to be reviewed here Some transaminases appear to be effective against several amino acids while others are more

## METABOLISM OF METHIONINE

In contrast to the branched chain amino acids methionine has two major metabolic pathways available to it Methionine transaminates to give α keto-α methiolbutyric acid [8] but to the authors knowledge the extent of further degradation is not known Methionine is converted to other amino acids by *enzymatic reactions involving sulfur*

The conversion of methionine to cystine was demonstrated unequivocally by isotope experiments [20 21] Homocysteine [22] serine [23 24] and cystathionine [25] were shown to be intermediates The sequence is as follows (Reaction 8)



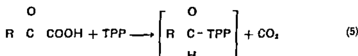

Methionine is converted to homocysteine in an enzymatic reaction which utilizes adenosine triphosphate [26] to reduce the energy barrier to transmethylation Several methyl acceptors are known A common one is guanidoacetic acid which is eventually converted to creatinine Homocysteine may be reconverted to methionine with betaine acting as the methyl donor Homocysteine and serine condense to form cystathionine which in turn cleaves to cysteine and homoserine It can be seen that serine is the sulfur acceptor The condensation and cleavage require different enzymes both requiring pyridoxal phosphate as a cofactor The oxidation of cysteine to cystine occurs spontaneously but the enzymatic conversion is also known [27] Further metabolic pathways of cysteine and cystine are apparently of no importance in the disease

isovaleric acid probably through α ketoisocaproic acid Coon and Abrahamsen [15] have presented similar indirect evidence that α methyl butyrate is an intermediate in the metabolism of isoleucine and they postulate that α keto β methylvalerate is an intermediate in the conversion

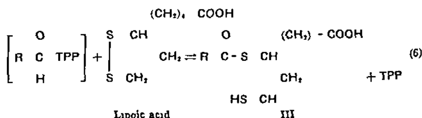
Leucine 1 $C^{14}$ is readily decarboxylated by rat brain liver, and kidney [4] Rabbit white blood cells obtained from peritoneal exudate are also effective but rabbit and human whole blood give negative results

The details of this complex enzymatic process have been deduced from studies with bacteria [16] but there is evidence that the pathway is similar in animal tissue [17] In the reactions to be described pyruvate and α ketoglutarate were used as substrates Although preliminary unpublished work suggests that the branched chain α keto acids behave similarly [18] definite conclusions are not warranted at this time

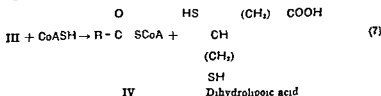
Several enzymes and cofactors are involved in the decarboxylation of α keto acids [16 19] Thiamine pyrophosphate (TPP) in a reaction not clearly understood catalyzes the elimination of carbon dioxide and becomes bound to a fragment which is probably at the aldehyde oxidation level (Reaction 5)



Another essential cofactor lipoic acid which is tightly bound to the enzyme oxidatively displaces TPP (Reaction 6) A bond forms between dihydrolipoic acid and the decarboxylated acid (III)



Coenzyme A (CoASH) displaces dihydrolipoic acid to produce an acyl CoA compound (IV) (Reaction 7) Diphosphopyridine nucleotide (DPN) and flavin adenine dinucleotide (FAD) are two more cofactors Further degradative pathways are reviewed elsewhere [28]



(Reactions 5 to 7) The first two acids were sought in the urine with gas phase chromatography None of the urinary peaks coincided with isovaleric acid (Table 15 1) but one did correspond with isobutyric acid However the area underlying the peak was considerably less than that of the keto acids The reverse would be expected if the metabolic block were beyond isobutyric acid The metabolic block is therefore presumed to exist between the keto acids and the simple acids and at the point of oxidative decarboxylation of the keto acids

TABLE 15 1 GAS PHASE CHROMATOGRAPHY OF ORGANIC ACIDS IN PATIENT'S URINE

| *Peak* | *Retention time* | *Compound* | *Percentage of total* |
|---|---|---|---|
| 1 | 5 | | 4 1 |
| 2 | 5 6 | α Ketoisovaleric acid ( ketovaline ) | [illegible] |
| 3 | [illegible] 8 | α Keto β-methylvaleric acid ( ketoisoleucine ) | 55 |
| 4 | 8 7 | α Ketoisocaproic acid ( ketoleucine ) | 8 |
| 5 | 13 1 | α Hydroxyisovaleric acid ( hydroxyvaline )<br>α Hydroxy β-methylvaleric acid ( hydroxyisoleucine )<br>α Hydroxyisocaproic acid ( hydroxyleucine ) | 5 |
| 6 | 22 5 | | 0 4 |
| 7 | 26 9 | | 0 [illegible] |

The acids were converted to the methyl esters and passed in the gas phase over polydiethylene glycol succinate

An unsuccessful attempt was made to demonstrate the postulated aldehyde intermediate (Reaction 5) If it were present in excess and in a form capable of reacting with 2 4-dinitrophenylhydrazine it should have the solubility characteristics of a neutral hydrazone No such compound could be demonstrated in the urine This observation is obviously open to more than one interpretation

Mackenzie and Woolf [5] have reported an excess of the branched chain amino acids and keto acids in the urine of Girl B It seems most probable that there is a similar metabolic block in their patient

The specific involvement of the three branched chain keto acids indicates that there is a common step in their metabolic pathways and suggests that an enzyme associated with their further degradation is deficient It is well to keep in mind that alternative theories can be constructed to fit the currently available data This aspect and the need for further study are discussed below in the appropriate section

## OTHER METABOLITES

The organic acids of the urine of Boy A were extracted and converted to the methyl esters for analysis by gas phase chromatography Seven

## THE 'METABOLIC ERROR'

In the investigation of Boy A by Westall et al amino acid analysis of the urine by column chromatography revealed increased excretion of leucine isoleucine, and valine There was also roughly a tenfold increase in the plasma concentrations of the branched chain amino acids (Fig 15 1) This indicated that the aminoaciduria was of the 'overflow' type

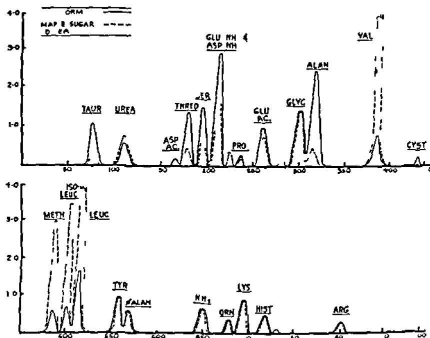
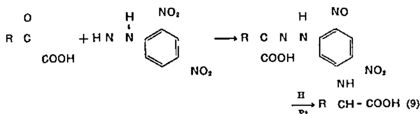



Fig 15-1 Plasma amino acid pattern determined by column chromatography The patient's levels (broken line) are compared with those of a normal subject (continuous line) [9]

and suggested a metabolic block in the degradative pathway of these amino acids Other abnormalities were noted in the plasma amino acid pattern particularly a high methionine and low cystine level However the elevations in the branched chain amino acids were more striking and therefore this aspect has received primary attention

Tissues obtained from Boy A at autopsy were able to transfer the amino group from the branched chain amino acids to $\alpha$ ketoglutarate (Reaction 2) indicating that a block lay at a lower level This was confirmed when an excess of $\alpha$ keto acids was found in the urine by paper chromatography [29] and by gas phase chromatography [4]

The next metabolic step is normally that of oxidative decarboxylation of the keto acids to isobutyric isovaleric and $\alpha$ methylbutyric acids




The keto acids can also be identified by paper chromatography of the 2,4-dinitrophenylhydrazone derivatives [30] and by gas phase chromatography of the methyl ester derivatives. For the last two methods pure samples of the keto acids are needed as standards. These are difficult to obtain commercially but can be synthesized enzymatically [31].

The demonstration that the simple branched acids isobutyric, isovaleric and α-methylbutyric acids are not present in excess will complete the diagnostic work up and localize the defect as accurately as is possible at present.

## METHODS

*Qualitative Demonstration of Excess Keto Acids*

1. To 1 ml urine add 4 ml 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid. A yellow precipitate forms.
2. After 10 min extract the precipitate with ether.
3. Extract the ether with 10 per cent sodium carbonate. In a positive test the yellow color enters the water phase.

*Identification of the Keto Acids [30]*

1. Begin as above but use a larger aliquot of urine (about 20 ml).
2. Acidify the sodium carbonate extract and extract with peroxide-free ether.
3. Distill off the ether and replace with 30 ml water.
4. Hydrogenate the mixture until it is colorless. This can be done in a hydrogenator over 75 mg activated platinum. An electrolytic desalter will accomplish the same result.
5. Identify the reconstituted amino acids by paper chromatography.

*Quantitative Analysis for Keto Acids [32]*

1. To 1 ml urine add 5 ml 15 per cent trichloroacetic acid and 500 mg Lloyd's reagent, *Zeolite or Permutit*. *Shake and centrifuge.*
2. To a 1 ml aliquot of supernatant add 0.2 ml 2,4-dinitrophenylhydrazine solution and mix. Let stand 15 min.
3. Add 1.5 ml *m*-xylene. Mix vigorously over 2 min period. Centrifuge.
4. To a 1 ml aliquot of the supernatant add 1.5 ml 10 per cent $Na_2CO_3$. Mix vigorously and centrifuge. Discard the upper phase.
5. To 1 ml of lower layer add 1 ml of 1.5 N NaOH. After 10 min read at 520 mμ.

For blank use 1 ml water instead of urine.

peaks were demonstrated Three had the same retention times as standard samples of the branched chain keto acids A fourth peak coincided with two of the *α hydroxyacid derivatives* of the branched chain amino acid The third hydroxyacid overlay one of the keto acids (Table 15 1) Three of the peaks were not identified Thus there is presumptive evidence for the presence of the hydroxyacid Mackenzie and Woolf have stated that there were "non keto acids with the properties of α hydroxy acids in the urine of Girl B but no further details are given in their report

*Indoleacetic* and *indolelactic acids* have been identified by paper chromatography in the urine of Boy A and Girl B and appear to be present in excess amounts

The compound or compounds responsible for the maple syrup odor is not known

## DIAGNOSIS

A clinical diagnosis is suggested by an infant presenting with central nervous system symptoms (vomiting poor feeding muscular hypertonicity) beginning possibly a week after birth and with a maple syrup odor to the urine Mental retardation rapidly becomes evident Variants of this clinical picture may be recognized with further experience If Menkes original patients suffered from the same metabolic anomaly as Boy A and Girl B then the disease varies from the acutely lethal to one compatible with existence for at least 2 years A second infant with this disease has been born to the family of Boy A confirming the familial nature of the disease The family of Boy A gave a definite history of waxing and waning of the maple syrup odor at times the odor could not be detected at all The diagnosis must therefore be suspected even in the absence of odor In this preliminary state of knowledge it would be wise to test for ketoaciduria (see below) in all cases of suspected congenital metabolic disease

*Amino acid analysis* of the urine or plasma may reveal an excess of the branched chain amino acids The plasma methionine may also be elevated The urinary levels of the branched chain amino acids in Boy A were normal at 10 months of age even though the branched chain keto acids were significantly elevated

A *ketoaciduria* may be demonstrated quantitatively or qualitatively The 24 hr excretion in Boy A was 49 mg and 252 mg at 6 months and 18 months of age respectively This contrasts with figures from three normal infants of 4 2, and 3 mg per 24 hr

The demonstration of ketoaciduria is useful as a screening test In order to establish further the diagnosis the keto acids must be identified as derivatives of the branched chain amino acids This is best done by converting the keto acid to the hydrazone which is reduced to the parent amino acid and then identified by paper chromatography (Reaction 9)

in the conversion of methionine to cystine There were also considerably reduced levels of other amino acids (Fig 15-1) The relation to the primary metabolic defect is not clear

The compound responsible for the maple syrup odor has not been identified The keto acids have no such odor but it may be due to a derivative or decomposition product In the case of Boy A, a reliable history was obtained that the maple syrup odor was not always present The authors have noted a similar odor in impure samples of fumaric acid pyruvic acid citric acid and $\alpha$ hydroxybutyric acid It is possible that a maple syrup odor may be caused by more than one compound or group of compounds and it is also possible that maple syrup urine diseases may be associated with other metabolic anomalies A recent report provides suggestive support for the hypothesis [33] The details of this case are not yet available but there was an increased excretion of the branched chain amino acids and phenylpyruvic acid and a burnt sugar odor to the urine

## GENETICS

The disease appears to be familial but its pattern is not yet clear Boy A was one of three children One sibling is normal and a second has just been born with the disease The parents are unrelated and no history can be obtained of a similar disease on either side Girl B is an only child and no significant genetic history is reported

## THERAPY

The possibility of treatment by a diet low in the branched chain amino acids is suggested by the success of low phenylalanine diets in phenylketonuria (Chap 10) Essential to the success of such an approach is that the infant be normal or at least not irreversibly damaged at birth There is so far no information on this aspect of maple syrup urine disease

## POSSIBILITY OF OTHER KETOACIDURIAS

Transamination is generally the first metabolic step in degradation of the amino acids and oxidative decarboxylation is commonly the second It would be surprising indeed if other ketoacidurias are not recognized eventually

## SUMMARY

Two unrelated infants have been reported with metabolic diseases characterized by early onset of feeding problems retarded development and a maple syrup odor to the urine In both instances the keto acids of leucine isoleucine and valine were found in the urine suggesting a block at the step of oxidative decarboxylation In one infant the plasma amino acids were investigated In addition to an elevation of the branched chain

*Reagents*

1 2 4 Dinitrophenylhydrazine solution Grind 100 mg of the compound Add 85 ml water mix and follow with 15 ml concentrated HCl Shake in shaking machine for 30 min Allow to settle and use supernatant

2 Standard Prepare 0 01 M standard solution in 0 1 N $H_2SO_4$ The branched chain keto acids are difficult to obtain commercially Pyruvic acid can be used Commercial samples frequently must be recrystallized The difference in extinction coefficients among these keto acids does not introduce a clinically significant error

## AREAS FOR FUTURE STUDY

### CLINICAL COURSE

At present there have been three reports of cases with a maple syrup odor to the urine associated with early signs of central nervous system disease More observations are needed to outline the natural history of the disease

### BIOCHEMISTRY

The primary metabolic anomaly can not yet be considered established The accumulation of the branched chain keto acids suggests a block at the next enzymatic step that of oxidative decarboxylation However similar findings could result from overloading a normal enzyme of limited capacity The high methionine and low cystine levels suggest another metabolic block This aspect requires further investigation It is possible that the primary anomaly lies in the metabolism either of methionine or of the branched chain amino acids or it may be that both anomalies have another as yet unidentified origin

A biochemical interpretation of the symptoms is lacking One may suspect that the symptoms arise from an excess of metabolites proximal to the block or derivatives of these No important metabolic function has been attributed to the compounds distal to the block

The preliminary identification of indole acids in the urine of maple syrup urine disease deserved further investigation In phenylketonuria it has been demonstrated that the indoleaciduria is associated with decreased formation of serotonin (5 hydroxytryptamine) and it has been suggested that this may be the primary factor in the mental deficiency Certainly similar studies should be done in maple syrup urine disease when possible It would be wise to keep in mind that the branched chain keto acids and hydroxyacids resemble certain essential normal metabolites and that their accumulation in excess might interfere with normal metabolism in more than one area

Other amino acid abnormalities in addition to the accumulation of the branched chain amino acid were noted in the plasma of Boy A The most striking was an elevated concentration of methionine (3 mg per 100 ml) and a level of cystine too low to be detected This suggests a block

26 Cantoni G L and Vignos P J Jr Enzymatic mechanism of creatinine synthesis J Biol Chem 209 647 1954
27 Romano A H and Nickerson W J Cystine reducta e of pea seeds an l yeasts J Bol Chem 208 409 1954
28 Meister A Biochemistry of the Amino Acids p 36 Acad mic 1 ess Inc New York 1957
29 Menkes J H Maple syrup d ease Isolation and identification of orgar c acids in the urine Pediatrics 23 348 1959
30 Meister A and Abendschein I A Chromatography of α-keto acid 4-d nitro phenylhydrazones and their hydrogenation products Analyt Chem 28 171 1956
31 Meister A Sodium alpha ketoisocaproate Biochem Preparations 3 66 1953
32 Natelson S Micro-techniqu s of Clinical Chem i t y fo the Rout ne Laboratory Charles C Thomas Springfield Ill 1957
33 Jepson J B Smith A J and Strang L B An inborn error of metabolism with urinary excretion of hydroxy acids keto acids and amino acids Lancet 2 1334 1958

amino acids the plasma methionine was high and the cystine low, suggesting that a block may also exist in this pathway Further study is needed to define the metabolic anomaly better

## BIBLIOGRAPHY


1 Menkes J H Hurst P L and Craig J M New syndrome progressive familial infantile cerebral dysfunction associated with unusual urinary substance Pediatrics 14 462 1954
2 Westall R G Dancis J and Miller S Maple sugar urine disease A M A J Dis Child 94 571 1957
3 Dancis J Levitz M Miller S and Westall R G Maple syrup urine disease Brit M J 1 91 1959
4 Dancis J Levitz M and Westall R G Maple syrup urine disease (branched chain keto aciduria) (in preparation)
5 Mackenzie D Y and Woolf L I Maple syrup urine disease an inborn error of the metabolism of valine leucine and isoleucine associated with gross mental deficiency Brit M J 1 90 1959
6 Blanchard M Green D E Nocito V and Ratner S L Amino oxidase of animal tissue J Biol Chem 155 421 1944
7 Cammarata P S and Cohen P P The scope of the transamination reaction in animal tissues J Biol Chem 187 439 1950
8 Needham D M A quantitative study of succinic acid in muscle-glutamic and aspartic acids as precursors Biochem J 24 208 1930
9 Meister A Transamination Advances Enzymol 16 185 1955
10 Green D E Leloir L F and Nocito V Transaminases J Biol Chem 161 559 1945
11 Meister A Sober H A and Peterson E A Studies on the coenzyme activation of glutamic aspartic apotransaminase J Biol Chem 206 89 1954
12 Braunstein A E Transamination and the integrative functions of the dicarboxylic acids in nitrogen metabolism Advances Protein Chem III 1 1947
13 Kinnory D S Takeda Y and Greenberg D M Isotope studies in the metabolism of valine J Biol Chem 212 385 1955
14 Bloch K Metabolism of leucine and valine J Biol Chem 155 255 1944
15 Coon M J and Abrahamsen N S B The relation of α methylbutyrate to isoleucine metabolism J Biol Chem 195 805 1952
16 Gunsalus I C Oxidative and transfer reactions of lipoic acid Fed Proc 13 715 1954
17 Sanadi D R Littlefield J W and Bock R M Studies on α ketoglutaric oxidase II Purification and properties J Biol Chem 197 851 1952
18 Dolin M I Unpublished data quoted in Fed Proc 13 715 1954
19 Koike M and Reed L J On the mechanism of oxidative decarboxylation of pyruvate J Am Chem Soc 81 505 1959
20 Tarver H and Schmidt C L A The conversion of methionine to cystine experiments with radioactive sulfur J Biol Chem 130 67 1939
21 Reed L J Cavallini D Plum F Rachele J R and du Vigneaud V The conversion of methionine to cystine in a human cystinuric J Biol Chem 180 783 1949
22 Brand E Cahill G F and Block R J Cystinuria—IV The metabolism of homocysteine and homocystine J Biol Chem 110 399 1935
23 Stetten D Jr The fate of dietary serine in the body of the rat J Biol Chem 144 501 1942
24 Binkley F and du Vigneaud V The formation of cysteine from homocysteine and serine by liver tissue of rats J Biol Chem 144 507 1942
25 Rachele J R Reed L J Kidwai A R Ferger M F and du Vigneaud V Conversion of cystathionine labeled with sulfur to cystine in vivo J Biol Chem 185 817 1950

Part Four

# Diseases Characterized by Evidence of Abnormal Lipid Metabolism (The Lipidoses)

# Chapter 16

# Essential Familial Hyperlipidemia

---

*Donald S Fredrickson*

The term *lipidosis* has been used to describe almost any disturbance of lipid metabolism particularly one leading to abnormal accumulations of lipid in blood or in certain organs A *primary familial lipidosis* by definition should arise from a genetically transmitted abnormality in lipid metabolism Five currently recognized diseases appear to meet this definition These may be conveniently subdivided into two groups

1 Primary familial lipidoses characterized by hyperlipidemia (the essential hyperlipidemias)
   - *a* Essential familial hyperlipemia
   - *b* Essential familial hypercholesterolemia

2 Primary familial lipidoses characterized by intracellular accumulation of sphingolipids (the sphingolipidoses)
   - *a* Sphingomyelinosis (Niemann Pick disease)
   - *b* Gangliosidosis (infantile amaurotic family idiocy)
   - *c* Cerebrosidosis (Gaucher s disease)

Each of these conditions represents a more or less distinct clinical syndrome that usually can be accurately diagnosed A well understood biochemical lesion has not yet been demonstrated for any of them however and it is possible that several specific diseases not all necessarily familial will eventually be separated from within each syndrome It is also possible that the inheritable abnormality in some of them may yet prove to involve the metabolism of protein or carbohydrate rather than lipid

A number of lipidoses have obviously been excluded from the above classification Many of these are lipidoses secondary to other diseases such as xanthomatous biliary cirrhosis the nephrotic syndrome or diabetes Others such as the hyperlipidemia seen in glycogen storage disease may be secondary to other inheritable molecular disease The

when more specific diagnosis cannot be made [1] When appropriate the type of xanthoma present may be added as a subordinate diagnosis

## BLOOD LIPIDS AND LIPOPROTEINS

Adequate de cription of hyperlipidemia depends upon the definition of the normal state of lipids and lipoproteins in blood and extracellular fluid

### BLOOD LIPIDS

In plasma from a normal human being there are usually *450* to *750* mg per 100 ml lipids (Table 16 1) Proper examination of the lipid content should be done on a pecimen collected after an overnight (12 to 16 hr) fast Either plasma or serum may be used Either should be perfectly

TABLE 16-1 NORMAL SERUM LIPID CONCENTRATIONS IN THE FASTING HUMAN BEING

| *Lipi l* | *Co ce t ation mg/100 ml* |
|---|---|
| Cholesterol (60–75% esterified) | 150–240 |
| Pho pholipi ls approximate con po ition—lecithin ( 0%) sphin gomyelin ( 0%) lysolecithin cephalin inos tol pl o pl ati le others (10%) | 150 ?50 |
| Glyceri les | 50–1 5 |
| Cerebrosi les | 3–6 |
| Carotenoids total | 0 1–0 . |
| Unesterified fatty acids | 0 3–0 6 (mE q/l) |
| Total fatty acids cholesterol e ter ( 0%) pho pholipi l (50%) glyceri le (?5%) une te fied (5%) | 200–400 |

clear to transmitted light if the patient has properly fasted If even faint lacte cence (hyperlipemia) is visible ignificant elevation in glyceride concentration is almost certainly present Some hyperglyceridemia may be present however in perfectly clear serum

#### *Chemical Analyses*

By far the mo t widely determined blood lipid is chole terol for which a number of u eful te ts have been developed The tandard of reference for all these i till the reliable Schoenheimer Sperry method [4]

Unless obvious hepatic di ea e is pre ent there is little to be gained however by separate mea urement of free and e terified chole terol in tudying hyperlipidemia Determination of total pho pholipids and

[1] In th St nda d Nom clat e of Disea es sponsored by the American Med l Associ t on [ ] only f tial hypercholestere ta i l ted The 19 ed t n of the Int at al Stat t l Cla fication of Dis ases ponso ed by th World Health Organ t n l ts only the unsatisf tory terms xantho atosis an l l p dos s f r classification of these yndromes [5]

"cholesterol lipidosis" represented by eosinophilic granuloma or the Hand-Schüller-Christian syndrome is nonfamilial and cannot be included either.

## DEFINITIONS

It is with misgivings that discussion of a subject already burdened with confusing terminology is begun with the introduction of another new term. *Essential hyperlipidemia* seems an appropriate generic term, however, for the group of lipidoses whose distinguishing feature is the presence of abnormally high concentrations of lipid in the extracellular fluid.

Sound argument could be advanced for the term *Essential hyperlipoproteinemia* since concentrations of some lipoproteins can be elevated without recognizable hyperlipidemia (*1*) but too few physicians have the facilities to measure lipoproteins, and lipid analyses alone are usually adequate for the purposes of diagnosis.

Unfortunately, the perfectly good term *hyperlipemia* has too long been used in a restricted sense to permit return to its broader meaning. In keeping with tradition it must be used to define lactescence of plasma due to hyperglyceridemia. *Essential* will be used here in place of *primary* or *idiopathic*. The weight of historical tradition demands also that the term *essential hypercholesterolemia* be retained to define a more or less distinct clinical syndrome, even though in this condition the phospholipid concentrations are almost always significantly elevated and often the glyceride levels are as well.

Much confusion can be avoided if the distinction between hyperlipidemia and xanthomatosis is maintained. Not all patients with essential hyperlipidemia develop xanthomas and the latter are most probably always a secondary manifestation dependent upon the hyperlipidemia. To emphasize the primary importance of the blood lipid changes, the term xanthomatosis should be subordinated.

It cannot be assumed that all cases of essential hyperlipidemia spring from an inheritable disorder of lipid metabolism. The proper concern here is only with those which appear to be genetically transmitted. These may be considered in terms of two clinical syndromes: *essential familial hyperlipemia* (synonyms: idiopathic familial hyperlipemia, hépato-splénomégalie lipoidose—type Bürger-Grütz) and *essential familial hypercholesterolemia* (synonyms: familial hypercholesterolemic xanthomatosis, primary hypercholesterolemic xanthomatosis, primary cholesterol lipidosis).

Since there are confusing cases of hyperlipidemia which have the features of both essential hypercholesterolemia and essential hyperlipemia (see below), it is suggested that until classification and diagnostic methods are improved the term *essential (familial) hyperlipidemia* be used

*Phospholipids*

The phospholipids and other complex lipids present more formidable chemical problems and exact analytical data for blood and tissue composition of many of the complex lipids are only slowly becoming available. Although it has been long known that most of the plasma phospholipids contained choline [*19*] recent use of silicic acid chromatography [*20*] has helped greatly in clarifying their composition. Lecithin (phosphatidyl choline) and sphingomyelin comprise almost 90 per cent of the plasma phospholipids. The remaining fraction has been reported to contain small

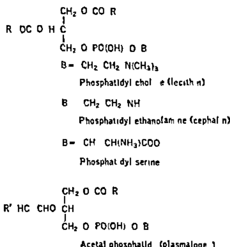



Fig 16-1 Some naturally occurring phospholipids. There is recent evidence [*110*] that R′ not R [ *] represents the saturated acyl group on lecithin

amounts of phosphatidyl ethanolamine lysolecithin phosphatidyl serine inositol phosphatide and possibly other minor amino-nitrogen-containing components. A small fraction of the phospholipids contain fatty acids as acetals.

In addition to the phosphorus containing complex lipids there are small quantities [*21 21a*] of cerebrosides sugar-containing sphingolipids.

It is believed that all naturally occurring lecithins (Fig 16-1) are of the L-α configuration. The same is probably true for phosphatidyl ethanolamine and other derivatives [*22*]. The specific acyl groups in each of the glycerophosphatides and their variation under different metabolic conditions remain to be established. Hanahan [*22*] has reported that the fatty acids in liver phospholipids are asymmetric, the unsaturated acid predominating in the α position and saturated acids in the β position. In plasma however more than half the phospholipid fatty acids may be unsaturated

glycerides requires no more complicated laboratory equipment but does demand technical skill especially in preparing quantitative lipid extracts which are free of nonlipid contamination In such an extract total lipids are usually determined gravimetrically or by reduction of an oxidant such as potassium dichromate [5] Total fatty acids may also be determined after saponification by titration or by weight or total carboxylic esters may be measured colorimetrically [6] Total phospholipids are usually determined by phosphorus analyses The values are expressed as pho pholipid by using a commonly accepted mean value for phosphorus as 4 per cent of the total weight of the phospholipids [7]

*Glycerides are most frequently determined as the residual lipid or fatty* acids not specifically accounted for by pho pholipid or chole terol For this reason normal values given for glyceride vary greatly in different laboratories In the author s clinic, using the methods of Bragdon [5] which measure total oxidizable material in a chloroform methanol water extract of serum one rarely sees glyceride values above 100 mg per 100 ml in a normal individual of any age More specific chromatographic separations of plasma lipids are available [8] but are too time-consuming for routine use

Of published methods for determining plasma unesterified fatty acid the method of Dole [9] is simplest but is still too complicated for wide clinical application

Diurnal variations in pla ma cholesterol and phospholipid levels may be ignored from the clinical standpoint Glyceride concentrations may vary many fold the highest concentrations occurring 3 to 5 hr after ingestion of fat Unesterified fatty acid concentrations are subject to very wide variations in normal individuals They are low after carbohydrate feeding and elevated during fasting exercise or emotional stress [9, 10]

*Cholesterol*

Cholesterol ($\Delta^5$ cholestene-3$\beta$-ol) is for all practical purposes the sole sterol in blood Cholestanol (cholestane 3$\beta$ ol) may be present in 1 to 3 per cent of the concentration of chole terol [11] Only 20 to 30 per cent of cholesterol molecules in plasma are the free alcohol In the rest the single hydroxyl is joined through an ester linkage with a fatty acid The e acids are largely unsaturated [12] and in man may be over 80 per cent linoleic and oleic acids [13] Except in clinically evident hepatic disea e the ratio of esterified to total cholesterol is remarkably constant [14] In certain of the essential hyperlipidemias some variation in this ratio may also occur thi will be explained later Although the biologic functions of chole terol are poorly understood an enormous amount of information has been accumulated concerning its biochemi try This information has recently been assembled in several texts [15 16] and reviews [17 18] to which the reader is referred for many details

UFA circulate bound largely to albumin which contains sites capable of firmly binding several moles of UFA per mole of protein [31] Small and possibly variable amounts of UFA also probably circulate bound to plasma lipoproteins [32] and possibly to erythrocytes [33 34] The chemical composition of the plasma UFA is now being gradually defined [35 36] but has not yet been determined under a variety of conditions As determined by vapor phase chromatography the number of acids normally present in human plasma is large It includes the saturated acids from $C_{10}$ to $C_{18}$ and significant amounts of long chain polyethenoid acids greater than $C_{18}$ in length Palmitic and oleic acids make up the largest components Short term feeding experiments indicate that the plasma UFA are not greatly affected by the composition of the fat in the diet [36] Their composition resembles the depot fats [37] but is somewhat higher in certain polyethenoids

The evidence indicating the role of UFA in plasma transport to meet caloric demands has accrued from a number of experiments recently summarized elsewhere in detail [38] *The plasma concentration rises* sometimes three to five-fold at times when requirements for oxidizable fat rise such as in fasting uncontrolled diabetes hyperthyroidism or exercise Supplying nonfat calories or restoring the capacity to oxidize glucose by giving insulin to diabetic patients quickly causes the plasma UFA concentration to fall [9 10] It has been shown that UFA are avidly extracted by tissues when there is need to burn fat [39] Studies with labeled fatty acids in man indicate that despite the tight binding to albumin the UFA leave the plasma at turnover rates varying from 0 1 to 2 mEq per min [40 41]

The bulk of plasma UFA probably arises from the adipose tissues and is oxidized in liver heart skeletal muscles and most other tissues with the possible *exception of the brain All available evidence indicates that* utilization of UFA is mainly governed by controlling their release from the adipose tissue depots [38] The mechanism of release has not yet been fully elucidated It appears to be related intimately to some critical amount of carbohydrate utilization and possibly to certain humoral and neurogenic factor Epinephrine norepinephrine [42] and ACTH [43] have been demonstrated to cause UFA release from isolated adipose tissue Most of these factors also cause the plasma UFA level to rise in the intact man or animal Growth hormone also elevates plasma UFA concentrations [44] Ganglionic blockade may prevent the UFA rise after psychic stress It is possible that the neurogenic stimulus is more primitive and separate from carbohydrate utilization as a regulator

*Carotenoids*

Carotenoids are aliphatic or alicyclic pigments usually containing 40 carbon atoms (8 isoprene units) Both hydrocarbons (carotenes) and

Lipsky et al [13] found 65 per cent linoleic and oleic acids in the plasma phospholipids in man

In lysolecithin one of the hydroxyl groups of glycerol is not esterified The significance of these compounds in plasma is not known but it is of interest that this phospholipid has been reported to comprise the bulk of a small amount of lipid that is associated with protein in molecules of greater density than all other plasma lipoproteins [23] (see below)

The acetal phospholipids called *plasmalogens* by their discoverers Feulgen and Bersin [24] are only a very minor constituent of plasma phospholipids The structure for them shown in Fig 16-1 is that proposed by Rapport et al [25] and Debuch [26] The $\alpha$ $\beta$ unsaturated ether linkage joining the fatty aldehyde to glycerol occurs predominantly at the $\beta$ position in ox heart plasmalogens [27] but may depend upon the species from which the tissue is obtained [28] Plasma acetal levels have been studied under different conditions but no specific relationship of acetal to hyperlipidemia has yet been reported Since they account for 40 per cent or more of the phospholipid in the heart [27] and possibly in other tissues they may ultimately prove to have considerable physiologic importance

The sphingolipids have a singular importance in the lipidoses characterized by intracellular accumulation of lipid They are discussed in detail in Chaps 17 to 19

*Glycerides*

Triglycerides are of great utility to the body comprising the major storage form of fatty acids in the adipose tissue and the principal form in which exogenous fatty acids are transported from the intestine to the plasma They may also be important in the net transport of endogenous fatty acids in the plasma but this has not been rigidly demonstrated experimentally The relatively simple structure of the triglycerides belies the work yet to be done on fixing the nature and arrangement of the fatty acid moieties found in glycerides in various tissues and in the blood under a variety of circumstances

Although the bulk of plasma glycerides are triglycerides [8] small quantities of di and monoglycerides have been reported during both fasting and fat absorption [29 30] The general term *glyceride* may be more proper in discussing plasma lipids whenever distinction between higher and lower forms is not made

*UFA*

The plasma unesterified (or nonesterified) fatty acids (UFA or NEFA) are almost negligible in concentration (Table 16-1) but may be more important than all the other lipids combined as the transport form of fat readily available for oxidation

UFA circulate bound largely to albumin which contains sites capable of firmly binding several moles of UFA per mole of protein [31] Small and possibly variable amounts of UFA also probably circulate bound to plasma lipoproteins [32] and possibly to erythrocytes [33 34] The chemical composition of the plasma UFA is now being gradually defined [35 36] but has not yet been determined under a variety of conditions As determined by vapor phase chromatography the number of acids normally present in human plasma is large It includes the saturated acids from $C_{10}$ to $C_{18}$ and significant amounts of long chain polyethenoid acids greater than $C_{18}$ in length Palmitic and oleic acids make up the largest component Short term feeding experiments indicate that the plasma UFA are not greatly affected by the composition of the fat in the diet [37] Their composition resembles the depot fats [37] but is somewhat higher in certain polyethenoid

The evidence indicating the role of UFA in plasma transport to meet caloric demands has accrued from a number of experiments recently summarized elsewhere in detail [38] The plasma concentration rises sometimes three- to five-fold at times when requirements for oxidizable fat rise such as in fasting uncontrolled diabetes hyperthyroidism or exercise Supplying nonfat calories or restoring the capacity to oxidize glucose by giving insulin to diabetic patients quickly causes the plasma UFA concentration to fall [9 10] It has been shown that UFA are avidly extracted by tissues when there is need to burn fat [39] Studies with labeled fatty acids in man indicate that despite the tight binding to albumin the UFA leave the plasma at turnover rates varying from 0 1 to 2 mEq per min [40 41]

The bulk of plasma UFA probably arises from the adipose tissues and is oxidized in liver heart skeletal muscles and most other tissues with the possible exception of the brain All available evidence indicates that utilization of UFA is mainly governed by controlling their release from the adipose tissue depots [38] The mechanism of release has not yet been fully elucidated It appears to be related intimately to some critical amount of carbohydrate utilization and possibly to certain humoral and neurogenic factors Epinephrine norepinephrine [42] and ACTH [43] have been demonstrated to cause UFA release from isolated adipose tissue Most of these factors also cause the plasma UFA level to rise in the intact man or animal Growth hormone also elevates plasma UFA concentrations [44] Ganglionic blockade may prevent the UFA rise after psychic stress It is possible that the neurogenic stimulus is more primitive and separate from carbohydrate utilization as a regulator

*Carotenoids*

Carotenoids are aliphatic or alicyclic pigments usually containing 40 carbon atoms (8 isoprene units) Both hydrocarbon (carotenes) and

oxygenated carotenoids (xanthophylls) are present in human plasma and tissues These nonpolar compounds depend upon solubility in fats or protein binding for transport and in plasma are found mainly with the low density lipoproteins Hyperlipidemia is commonly accompanied by hypercarotenemia This is conceivably because of increased absorption or mobilization of carotenoids as the amounts of lipoprotein vehicle in plasma are increased Carotenoids contribute the yellow color to xanthomas and atheromas that are frequently associated with hyperlipidemia Among the more recent chemical studies of carotenoids in human tissues are those of Blankenhorn [44a] A single case of hypercarotenemia attributed to an inborn defect in conversion of carotenoids to vitamin A has been reported [44b]

*Lipoproteins*

The lipids in plasma and other extracellular fluid are solubilized and made available for transport and biochemical reactions by virtue of their linkage with protein Except for UFA, which bind primarily to albumin all the other lipids in plasma are bound to certain of the plasma globulins in the form of lipoproteins The structural specificity and unique metabolic properties of these macromolecules appear to warrant considering them distinct biochemical entities

At present lipoprotein analyses do not add essential information beyond that obtained by chemical determination of blood lipids in diagnoses of the hyperlipidemic states It has not been excluded however that the biochemical lesions in certain hyperlipidemias might be defects in lipoprotein metabolism as distinguished from defects in metabolism of only the lipid moieties

The initial discovery of a lipid protein complex in plasma was made by Macheboeuf in 1929 [45] As a result of impressive effort by many investigators a broad spectrum of lipoproteins has been discovered in extracellular fluid Present understanding of these lipoproteins is derived mainly from the chemical composition and metabolism of certain groups of molecules as rather arbitrarily defined by the methods used to isolate them

**Isolation** The ordinary extraction of plasma with most fat solvents destroys the stability of lipoproteins by either dehydrating the molecules or denaturing the protein moieties This makes it necessary to isolate the lipoproteins by gentler methods based on certain of their physical properties These methods include electrophoresis precipitation in media of high ionic strength and ultracentrifugation The most versatile of the present methods utilize the low densities of the lipoproteins to separate them from the other plasma proteins and to effect separation within the lipoproteins themselves

Gofman and his colleagues [46–48] have pioneered in developing

methods for using the ultracentrifuge to segregate lipoproteins according to their densities Several useful modifications of their technique are available for separating broad groups of lipoproteins in quantities sufficient for chemical analysis [49 50] In these method concentrated salt solutions are added to plasma to achieve a desired density and the solution is centrifuged in fields which may exceed 100 000 × gravity In this way the lipoproteins lighter than the density of the medium are concentrated at the top of the centrifuge tube and may be selectively removed for quantitation This is accomplished by actual analyses of lipid and protein content or by recentrifugation in an analytical ultracentrifuge The latter is equipped with an optical system through which concentration gradients created by floating lipoproteins may be seen and photographed The concentrations of these subgroups or density classes are obtained by measuring the area beneath the migrating peaks

According to the method of Lindgren Elliott and Gofman [46] the flotation rates of lipoproteins of density less than 1 063 are assigned values expressed in $S_f$ units (Svedberg units of flotation in $10^{-13}$ cm/ cc/ dyne/gm) The $S_f$ values of lipoproteins vary directly with their speed of flotation a property inversely dependent upon the density So-called standard $S_f$ units [47] refer to values corrected for temperature and for the effects of over all lipoprotein concentration on the flotation rates of a given class

It is most likely that a single lipoprotein system exists for all extracellular fluid even though the concentrations of most lipoproteins tend to be somewhat higher in plasma than in lymph The lipoproteins represent a density spectrum from about 0 9 to 1 2 gm per ml Within this spectrum they may be most conveniently divided into three large groups each capable of some further subdivision These major groups are the high density lipoproteins and low density lipoproteins (according to the Gofman terminology) and the chylomicrons

**High density Lipoproteins** The high density lipoproteins isolated by ultracentrifugation between densities 1 21 and 1 063 are also known as $\alpha_1$ lipoproteins because of their migration with the $\alpha_1$ globulins on electrophoresis In the analytical ultracentrifugal technique of deLalla et al [47] they are not assigned $S_f$ values but have been subdivided into groups designated the $HDL_1$ $HDL_2$ and $HDL_3$ lipoproteins which increase in density in this order Concentrations of these subgroups change independently under certain conditions [48]

**Low density Lipoproteins** Low density lipoproteins are floated in the ultracentrifuge at density 1 063 As indicated above these lipoproteins may be assigned $S_f$ values when the analytical ultracentrifuge is used Their concentrations are often measured in convenient groups such as for molecules lying between $S_f$ 0 to 10 10 to 20 20 to 100 and 100 to 400 Low density lipoproteins of $S_f$ 0 to 12 which float between densities

1 063 to 1 019, migrate with the $\beta$ globulins On starch block electrophoresis those above $S_f$ 12 migrate with $\alpha_2$-globulins [51] Hence the terms $\beta$ lipoproteins and $\alpha_2$-lipoproteins are often used in connection with low density lipoproteins Above $S_f$ 400 the low density lipoproteins merge without a sharp line of demarcation into the third arbitrary group of lipoproteins the chylomicrons

**Chylomicrons** Chylomicrons as they were first termed by Gage [52] are large (0 5 to 1 5 $\mu$) particles of emulsified fat associated with small but probably significant amounts of protein Normally present in lymph and plasma for only a few hours after the ingestion of fat they can be abundantly present in hyperlipemic conditions in the postabsorptive state

Since particles above about 0 1 $\mu$ scatter light increased concentrations of chylomicrons or the large low density lipoproteins cause lactescence in plasma These lipoproteins are also rich in triglyceride and lactescence (or hyperlipemia) invariably means hyperglyceridemia Light scattering is also a function of particle size and one cannot, therefore make a direct correlation between the degree of lactescence and the chemically determined concentrations of glycerides

**Other Plasma Lipoprotein** A small amount of lipid soluble phosphorus is present in the plasma fraction of greater density than 1 21 [49] It is not certain whether this represents lipoprotein of very high density or phosphoprotein It has recently been reported [23] that as much as 8 per cent of the plasma phospholipid may be present in this fraction about half as lysolecithin This is associated with other fatty acid ester but little or no cholesterol The significance if any, of this lipid in essential hyperlipidemia has not been explored

**Lipoprotein Composition** Table 16-2 illustrates the relationship between composition and density of the lipoproteins In normal individuals about one third of the plasma cholesterol is present in high density or $\alpha_1$ lipoproteins Most of the rest is present in low-density lipoproteins of $S_f$ 0 to 100 It will be observed (Table 16-2) that the proportion of cholesterol to phospholipid is much greater in the low density lipoproteins than in the high density lipoproteins *Essential hyperlipidemia is always characterized by elevation of one or more low density lipoprotein fractions and often by an elevated cholesterol to phospholipid ratio*

Specific differences in composition within the chemical classes of lipids in different lipoproteins need to be further explored The lipoproteins of density < 1 063 contain a higher molar ratio of sphingomyelin to lecithin and lower ratios of cephalin to sphingomyelin and lysolecithin to lecithin [54 55] compared with the high density lipoproteins

The nature of the lipid protein bond is not certain but it is loose enough to permit cholesterol and phospholipid molecules to transfer

Table 16-2 Composition of Several Fractions of Human Serum Lipoprotein

| Fraction | Density | $S_f$ (approximate) | Per cent cholesterol | | Composition | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Free | Ester | Phospholipid | Glyceride | Protein | FC/TC | TC/PL |
| Chylomicrons | <1 006 | >400 | 3 1 | 6 1 | 7 1 | 81 3 | 2 5 | 0 47 | 0 95 |
| Low-density lipoprotein | <1 019 | 12–400 | 6 0 | 16 2 | 17 9 | 51 8 | 7 1 | 0 37 | 0 90 |
| Low-density lipoprotein | 1 019–1 063 | 0–12 | 8 5 | 42 3 | 23 6 | 5 1 | 20 5 | 0 25 | 1 4 |
| High-density lipoprotein | 1 063 1 21 | | 2 3 | 18 5 | 26 9 | 4 6 | 47 | 0 17 | 0 50 |

Density of the medium in which the lipoproteins are floated in high centrifugal fields

Note FC = free cholesterol TC = total cholesterol PL = phospholipid

Source J H Bragdon et al [53] obtained by chemical analysis of fractions isolated in the preparative ultracentrifuge

readily from one lipoprotein to another [56, 57] Exchange of lipid also takes place rapidly between erythrocytes and plasma lipoproteins [58] Hence lipids may well enter tissues without taking the entire lipoprotein molecule with them

In contrast to the lipids the protein portions of the lipoproteins are not all exchangeable As determined by the chemical and immunochemical findings of many workers summarized elsewhere [38] the peptide residues of groups of lipoproteins of different densities are not the same Interconversion' of lipoproteins labeled in the protein moiety has been observed within the low density lipoproteins [59] but not between low density and high density lipoproteins [59 60] These two groups of lipoproteins may be quite unrelated in their physiologic function The relationship of chylomicrons to the other lipoproteins is not certain The small amounts of protein associated with these large particles (Table 16-2) include polypeptides identical with those in the high density lipoproteins [61]

It has been proposed that chylomicrons might be shorn of lipid and give rise to the other lipoproteins present in plasma [62] Defects at various stages of such transformations could then account for accumulation of abnormal amounts of various lipoproteins characteristic of different types of hyperlipidemia The evidence presently available does not adequately support this attractive hypothesis [38] although it may be valid for part of the lipoprotein spectrum [59] Several preliminary reports have described synthesis of lipoprotein by the liver [63–65] Since this organ contributes most of the cholesterol and phospholipid to the plasma this is a logical development Incorporation of labeled amino acids into chylomicron proteins by isolated dog intestinal mucosal cells has also been observed [66] The mechanisms and sites of lipoprotein formation may be keys to understanding hyperlipoproteinemia, but acquisition of this knowledge is confronted with many unusual technical difficulties

Xanthomatosis Essential hyperlipidemia is commonly recognized through one of two manifestations xanthomas or atherosclerosis Both involve accumulation of lipid in tissues and the mechanisms responsible have been under study and debate for many years No attempt will be made here to review the voluminous literature pertaining to these problems The weight of current evidence indicates that in the presence of hyperlipidemia xanthoma formation and accelerated development of atherosclerosis are secondary phenomena Abnormal amounts of lipid are taken up from extracellular fluid either because the lipids are there in excessive concentrations or because the factors responsible for their solubilization permit the equilibrium to shift in the direction of tissue deposition

The location and appearance of xanthomas in hyperlipidemia depend upon among other things the nature of the abnormal blood lipid pattern

Four or five xanthoma types are distinguished and will be mentioned in subsequent clinical de criptions of these syndromes There are xan thelasma tendon tuberous and plane xanthomas common to essential hypercholesterolemia and eruptive xanthomas which appear only in severe hyperlipemia Detailed de criptions of xanthomas appear in all standard dermatology texts and in Thannhauser s excellent monograph on the lipido es [21] A helpful clarification of the variable terminology used has been provided by Crocker [67] Literature concerning arcus senilis or corneal arcu [68] an ophthalmologic change sometimes associated with hypercholesterolemia has been reviewed by Klatskin [69]

## ESSENTIAL FAMILIAL HYPERLIPEMIA

Hyperlipemia when defined as an elevation of plasma glycerides above 100 to 150 mg per 100 ml in the fasting state is not rare although large cale studies fixing the normal limits in any population have not been made When defined as it u ually i as sufficient hyperglyceridemia to produce visible lactescence in plasma hyperlipemia is more uncommon but still not a rare phenomenon Since all hyperlipemia except that seen postprandially is essential in that the biochemical mechanism is not understood it is currently a very difficult task to classify satisfactorily the causes of this manifestation Several of the causes but certainly not all probably involve inherited defects in lipid or lipoprotein metabolism Of these hyperlipemia is associated mainly with the trait defined sub sequently as essential familial hypercholesterolemia In addition there is a smaller group of cases probably including more than one abnormal trait in which evere hyperlipemia is the outstanding inheritable feature These are grouped in the trait which has come to be known as essential familial hyperlipemia

### HISTORICAL ASPECTS

The historical development of essential familial hyperlipemia begins in 1932 when the first case was reported by Burger and Grutz [70] Sub sequently more than forty ca es have been reported in which the out *standing feature has been severe hyperlipemia closely related to the* intake of dietary fat The hyperglyceridemia has usually been accom panied by elevated concentrations of cholesterol and phospholipids but of a much less significant degree In 17 ca es involving 9 pedigrees the hyperlipemia was found to be familial [71–81] The authors reporting the first such familial case Holt Aylward and Timbres [71] coined the term *idiopathic familial hyperlipemia* Their patient a 12 year old girl was also the first to demonstrate the rare association of fever and ab dominal pain developing when the hyperlipemia became severe (total lipids about 8 gm per 100 ml) As the fever declined o did the hyper lipemia The liver and spleen grew larger as the hyperlipemia decreased

and the authors considered that the excess fat in the blood may have been taken up by these organs

Although not all the above forty-odd cases were shown to be familial they shared many common features In many there were associated eruptive xanthomas hepatosplenomegaly and bouts of abdominal pain In a very few glycosuria was observed but not diabetes Very infrequently pancreatitis developed after xanthomas or hyperlipemia had been present for some time [73 77, 82] In none did the initial report mention significant cardiovascular disease xanthelasma, tuberous, plane, or tendon xanthomas, or corneal arcus

In the past few years many cases of hyperlipemia have been reported as "essential hyperlipemia" which differ clinically from the more homogeneous features of the above cases These include many of the cases reported by Lever [83] Malmros [84] Schettler [85] and their colleagues Adlersberg [86] and others [80 87] Practically all these patients have been adults Many have had xanthelasma tuberous or even tendon xanthomas and evidence of severe coronary artery disease Some have had moderately severe diabetes [88 89] In a number of the relatives of probands hyperlipidemia has been present but usually hyperglyceridemia has been slight and the picture indistinguishable from essential familial hypercholesterolemia

Borrie [90] analyzing this entangled problem in 1957 concluded that most of the patients reported by Lever and Malmros had essential hypercholesterolemia and associated hyperlipemia He employed the term 'mixed cases' for that confusing group in which either diagnosis could reasonably be made In the third edition of his book Thannhauser [91] also recognized a group of patients with hyperlipemia in which he considers the primary disease to be essential hypercholesterolemia

Further confusion is offered by the recent interesting study of Hirschhorn et al [91] in which hyperlipemia was diagnosed in Swedish students by determination of the serum optical density in the fasting state and during oral fat tolerance tests Although their report does not describe studies in the families of the probands the authors concluded that the incidence of familial hyperlipemia in this population was between 2 and 3 per cent They define this condition as an inherited disease associated with early onset of coronary atherosclerosis and thought to be due to a single gene difference causing a defect in the lipemia clearing system

It is obvious that clinical distinction between the syndromes of essential hyperlipidemia has become increasingly arbitrary when significant hyperglyceridemia is also present It is necessary to adopt one of two assumptions Either essential hyperlipidemia is only one basic disease with many variants in the clinical pattern or there are several basic disorders with overlapping clinical features An argument for the possibility of a single

le ion associated with an entire spectrum of abnormal lipid and lipo protein patterns is provided by the variable manifestations of hyper lipidemia which may be seen in the nephrotic syndrome On the other hand the extreme manifestations of the clinical syndromes defined below and in the following section as essential familial hyperlipemia and essen tial familial hypercholesterolemia are very different and the majority of patients who appear to fall into one of these two groups do not show evidence of converting from one syndrome to the other The concept of multiple di orders is also supported by the observation of basic dif ferences within the group of patients who have e sential hyperlipemia indicating the pre ence of several varieties of this syndrome alone

Until better means of biochemical differentiation become available the classification of patients with lactescent erum not secondary to other di ease as having essential hyperlipemia is no doubt proper as long as it is recognized that semantic unity may hide biochemical heterogeneity

Because of present uncertainty the diagnosis and discussion of essential familial hyperlipemia will be somewhat arbitrarily restricted here to that small group of patients who e hyperlipemia and other manifestations resemble the dramatic features of the cases described by Burger and Grutz [70] and Holt et al [71] It is fully realized that lesser expression of the responsible abnormal traits may be present possibly in heterozygotes and account for many more or less dramatic cases not included in the discussion Although the hyperlipemia in this syndrome appears to be due primarily to defective removal from the plasma of exogenous fat in the form of chylomicrons the possibility of defective endogeneous glyceride metabolism will also be considered

## CLINICAL FEATURES

The clinical features of 17 cases of essential familial hyperlipemia in which familial occurrence has been demonstrated [71–81] appear in Table 16-3 About 25 other cases superficially similar but lacking known familial occurrence have been reported Most of these 25 cases are usually included in consideration of this syndrome and specific references to them appear in several reviews [83 92 93] That their inclusion is probably justified may be seen by comparison of their clinical features also tabulated in Table 16-3

There is a tendency for cases not shown to be familial to be detected later in life and several [21] have had some evidence of poor carbohydrate tolerance The latter has never been reported in a familial case unless pancreatitis was also present Largely because of this Thannhauser has arbitrarily divided this syndrome into childhood cases and adult cases with occasional glycosuria For reasons discussed below the author of this chapter has excluded a number of his own cases with moderate to

TABLE 16-3 CLINICAL FEATURES OF ESSENTIAL FAMILIAL HYPERLIPEMIA

| | Age at detection, yr | | Sex | | Enlarged liver | Enlarged spleen | Abdominal pain | Eruptive xanthomas | Plasma lipid mg/100 ml | |
|---|---|---|---|---|---|---|---|---|---|---|
| | < 1 | >21 | M | F | | | | | TG | TC |
| Known familial (17) | 1 | 5 | 13 | 4 | 10 | 10 | 8 | 4 | 3,100 | 460 |
| Not proved familial (26) | 14 | 1 | 20 | 6 | 17 | 16 | 11 | 11 | 4,200 | 434 |

NOTE Only f f th 43 has b n n by th th th r m f m th bt t (ce t t)

M n pl ma o ntr t n of t gly le (TG) and t t l ch l te ol (TC) f ll es h b th e v l w p ted

severe hyperlipemia and mild glucose intolerance from this classification They may well prove to represent a separate syndrome

*Detection*

The syndrome is likely to be discovered incidentally with the finding of milky serum on routine examination or, less frequently during clinical evaluation for hepatic or splenic enlargement or because of sudden development of eruptive xanthomas Rarely the hyperlipemia will be observed for the first time by an alert physician examining a patient who is in an acute "surgical emergency" with severe abdominal pain and signs of peritoneal irritation

The age of detection has varied from a few months to middle age About half the known familial cases were detected before age 6 and there undoubtedly is a tendency for childhood cases to have more severe manifestations Many patients appear in good health and may have no other abnormal finding than hyperlipemia It is probable that the syndrome is not so rare as the number of case reports suggests

*Plasma Lipids*

The serum is characteristically lactescent varying from moderate turbidity to the appearance of cream (which indeed it may approximate in total lipid content) Plasma lipid analyses for four familial cases studied in the author s clinic are shown in Table 16-4 Since the levels tend to fluctuate greatly in any one patient the values associated with the maximum total lipid concentrations obtained in a series of analyses are presented Total lipids of 18 gm per cent have been reported [94] The important features are triglyceride concentrations twenty to forty times normal associated with cholesterol and phospholipid concentrations increased by only two- to threefold More important than the absolute concentrations is the effect of dietary fat restriction This always causes

a rapid fall in all elevated plasma lipids the relative proportions between the several lipids remaining about the same The hyperlipemia sometimes seen in essential familial hypercholesterolemia may respond to the same treatment but the cholesterol and phospholipids will often remain disproportionately elevated

The frequent finding of a lower percentage of esterified cholesterol (Table 16-4) is no doubt related to a higher percentage of free cholesterol in the triglyceride-rich lower density lipoproteins characteristic of severe

TABLE 16-4 PLASMA LIPID ANALYSES IN FOUR PATIENTS WITH ESSENTIAL FAMILIAL HYPERLIPEMIA

| *Patient* | TL *mg/100 ml* | TG *mg/100 ml* | TC *mg/100 ml* | PL *mg/100 ml* | EC *per cent* | TG/TC |
|---|---|---|---|---|---|---|
| J P | 2 685 | 2 014 | ꞔ34 | 333 | 55 | 8 6 |
| I P | 3 5ı0 | 2 946 | 238 | 281 | 5˒ | 12 4 |
| P P | ꞔ 1ı0 | 4 61 | 363 | 31ı | 4ꞔ | 1? 0 |
| J L | 580 | 4 037 | 668 | 5ı˒ | 6ꞔ | 6 0 |
| Mean | 1 ?50 | 3 339 | 3ı6 | 376 | 5 | 9 8 |
| Normal | ı50 | 125 | 240 | ?50 | 70 | 0 4 |

Upper limits of normal for all age groups in the author s laboratory

NOTE TL = total lipids TG = triglyceride TC = total cholesterol PL = phospholipid EC = esterified cholesterol

hyperlipemia (Table 16-2) and is not an indication of hepatic disease Conventional liver function test results with the possible exception of a slightly elevated thymol turbidity are normal in essential familial hyperlipemia The presence of turbidity often makes the thymol test impossible to determine accurately

In a few patients studied so far [9 81] fasting UFA levels have not been recognizably abnormal

*Lipoproteins*

The lipoprotein changes in essential hyperlipemia are characterized by enormous increases in chylomicrons and low density lipoproteins of high flotation rates A comparison of the changes in a large number of lipoprotein classes as isolated ultracentrifugally may be seen schematically represented in Fig 16-2 Reciprocal decreases in high density lipoproteins particularly the $HDL_2$ fraction and sometimes in $S_f$ 0 to 20 low density lipoproteins are a common occurrence in severe hyper lipemia regardless of whether the latter is essential or secondary When the hyperlipemia is reduced the lower concentrations of these lipoproteins are usually but not always restored toward normal

The lipoprotein findings obtained by electrophoresis vary with the method used Paper electrophoresis will demonstrate great increases in

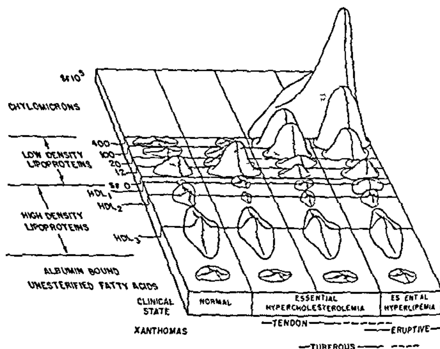



*Fig 16-2* Distribution of lipid in lipoproteins in essential hyperlipidemia Only the lipid moieties are represented as calculated from the data of Gofman et al [48] from the known lipid composition of the various fractions For emphasis what is actually a continuous spectrum of changes has been presented in three groups alongside the normal distribution The presentation does not conform to scale and the indicated association of xanthomas with various lipid patterns is only relative

the $\beta$ lipoproteins and a chylomicron tail will appear at the origin Starch block or moving boundary electrophoresis will reveal increases in the $\alpha_2$ peak and sometimes in the $\beta$ peak [95]

*Hepatosplenomegaly*

This has been observed in approximately two thirds of the cases discovered before age 6 and in about one-third of those detected later Only one organ or both may be enlarged The size of the organs may change greatly and in every case has been reported to decrease with institution of a low fat diet A possible cause of organ enlargement is accumulation of lipid although Thannhauser [21] disagrees with this hypothesis as far as the liver is concerned In the single autopsy reported for this disease by Chapman and Kinney [96] Thannhauser and Reichstein found that the liver fat content was not abnormal except for a possible decrease in sphingomyelin It is noteworthy that this child died of a severe intercurrent infection and the history suggests that it may have been malnourished at the time of death The liver contained no foam cells

but they were prominent in the spleen A case described by Rohn et al [97] in which the diagnosis of essential hyperlipemia cannot be made with certainty also came to autopsy The patient was a young female discovered to have marked hyperlipemia and hepatomegaly in the eighth month of pregnancy Phagocytic cells laden with fat were observed in liver and sternal marrow biopsies She died suddenly at term The liver was found to contain minimal fatty infiltration but the spleen was almost replaced by foam cells The cause of death was splenic and portal vein thrombosis

*Foam Cells*

In most cases foam cells may be found in biopsies of tissues rich in reticuloendothelial cells They are usually present in bone marrow and spleen and may appear in liver and lymph nodes These large histiocytes are 10 to 90 μ in diameter and contain droplets of lipid which give the cytoplasm a finely reticulated or mulberry appearance The droplets stain variably with Nile blue and Sudan III [96] but in the author s experience are Smith Dietrich negative Since the foam cells may very closely resemble the Niemann Pick cell (Chap 18) in over all appearance this latter staining reaction is of academic interest although the two diseases are easily separated by other considerations

The foam cells in essential hyperlipemia are identical to those early reported in the livers of diabetic patients [98 99] and no doubt arise by the same mechanism leading to xanthoma formation Presumably this is a phagocytic reaction of the reticuloendothelial system to the excessive fat in the blood and does not represent any increased synthesis of fat *in situ* Vacuolization of parenchymal as well as Kupffer and sinusoidal cells has also been observed in biopsy specimens from the liver [74]

*Xanthomas*

The characteristic skin xanthoma in essential hyperlipemia is of the eruptive type (Fig 16 3) These lesions characteristically appear when the hyperlipemia is severe and disappear when it is reduced by a low fat intake There is no predictable triglyceride level or duration of hyperlipemia at which lesions will appear The author has seen them appear in a patient with plasma triglycerides at only 2 000 mg per 100 ml for a short time and fail to develop in a young patient whose plasma triglycerides averaged 4 000 mg per 100 ml for over 6 years In several cases [70 71 100] small vesicles which have broken down and exuded milky fluid have also been described

By definition a number of cases of severe hyperlipemia with tuberous and tendon xanthomas have been excluded from the series of patients considered to have essential familial hyperlipemia It cannot be assumed dogmatically however that they never occur The clinical distinction

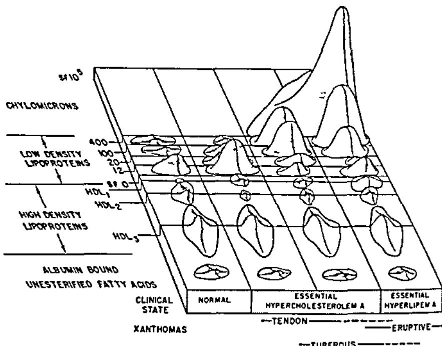



Fig 16-2 Distribution of lipid in lipoproteins in essential hyperlipidemia Only the lipid moieties are represented as calculated from the data of Gofman et al [48] from the known lipid composition of the various fractions For emphasis what is actually a continuous spectrum of changes has been presented in three groups alongside the normal distribution The presentation does not conform to scale and the indicated association of xanthomas with various lipid patterns is only relative

the $\beta$ lipoproteins and a chylomicron tail will appear at the origin Starch block or moving boundary electrophoresis will reveal increases in the $\alpha_2$ peak and sometimes in the $\beta$ peak [95]

*Hepatosplenomegaly*

This has been observed in approximately two thirds of the cases discovered before age 6 and in about one third of those detected later Only one organ or both may be enlarged The size of the organs may change greatly and in every case has been reported to decrease with institution of a low fat diet A possible cause of organ enlargement is accumulation of lipid although Thannhauser [21] disagrees with this hypothesis as far as the liver is concerned In the single autopsy reported for this disease by Chapman and Kinney [96] Thannhauser and Reichstein found that the liver fat content was not abnormal except for a possible decrease in sphingomyelin It is noteworthy that this child died of a severe intercurrent infection and the history suggests that it may have been malnourished at the time of death The liver contained no foam cells

It may be associated with spasm rigidity and rebound tenderness leukocytosis and fever There may be vomiting and collapse Other laboratory tests including those for serum amylase or lipase will usually give negative results and only the observation of grossly milky serum may spare the patient a needless laparotomy For excellent descriptions of these abdominal crises see the reports by Holt et al [71] and Bloomfield and Shenson [102]

The attacks of abdominal pain are rarely so far as can be determined at laparotomy associated with abnormality of the pancreas In fact there often is no visible abnormality save possible enlargement of liver or spleen The cause of pain has been thought to be distention of these viscera by uptake of lipid [71] The blood is not necessarily free of hyperlipemia at the height of the pain

*Pancreatitis*

Poulson [73] and Kennedy and Collett [82] have reported cases of familial hyperlipemia in which eruptive xanthomas or hyperlipemia have been observed well in advance of an attack of abdominal pain which proved at laparotomy to be associated with unquestionable pancreatitis Klatskin and Gordon [77] have also reviewed this problem and have presented several patients with similar convincing evidence that hyperlipemia can precede pancreatitis They postulated that fat embolization may cause this disorder but this has not been demonstrated experimentally The mechanism is not known It seems unlikely that all cases of acute pancreatitis which is not a rare disease should be due to essential hyperlipemia Why the former is often associated with transient hyperlipemia likewise remains an enigma

## DIFFERENTIAL DIAGNOSIS

The diagnosis of essential familial hyperlipemia depends upon the exclusion of any cause of secondary hyperlipemia and the hyperlipemia associated with essential familial hypercholesterolemia The nephrotic syndrome von Gierke s disease or the rare cases of hyperlipemia associated with myxedema xanthomatous biliary cirrhosis neoplasia hemorrhage and other obvious primary diseases present little difficulty Hyperlipidemia associated with pancreatitis or mild diabetes may offer confusion Unless hyperlipemia is known to have antedated either of these conditions the concomitant diagnosis of essential familial hyperlipemia should not be made Essential familial hypercholesterolemia with hyperlipemia is often associated with tuberous and tendon xanthomas and cardiovascular disease and there is a more pronounced familial involvement The predominantly elevated lipoprotein classes are often low density lipoproteins of high flotation rate rather than primarily chylomicrons This syndrome is discussed more fully further on in this chapter



Fig 16-3 Eruptive xanthomas on the buttocks of a patient with essential hyperlipemia These lesions completely disappeared after 4 weeks of very low fat intake.

between eruptive and tuberous xanthomas (Fig 16-8) is sometimes arbitrary Both terms have been used in the literature for isolated yellow plaques with an inflammatory base

*Lipemia Retinalis*

Lipemia retinalis occasionally offers a basis for detection of severe hyperlipemia This sign consists of a marked increase in light reflex of the retinal vessels and a pale cast to the fundus It has been stated [*101*] that a triglyceride concentration of over 2 000 mg per 100 ml is required for the appearance of lipemia retinalis but probably this is also a function of the particle size of the lipoproteins present Lipemia retinalis is no more specific a sign in essential hyperlipemia than is the incidental finding of milky fluid on a pleural tap for example Either simply reflects elevated glycerides and very low density lipoproteins in the extracellular fluid

Dunphy [*101*] and Falls (in discussing the former s paper), describe several cases with clouding of the cornea or lipid interstitial keratitis in association with hyperlipemia The exact nature of this symptom is obscure but it probably is due to some incidental process associated with transudation of lipid rich lymph

*Abdominal Pain*

Slightly less than half the patients with essential hyperlipemia have complained of episodic abdominal pain Frequently epigastric or mid abdominal the pain is often not localized and may radiate to the back

decreased by higher initial concentrations or by the injection of a previous load of chylomicrons [106] Although the behavior is said to be characteristic of removal of a substance by the reticuloendothelial system there is no certain evidence that these cells are more important than parenchymal cells in the removal of chylomicrons When isolated rat liver is perfused with chylomicrons more fat droplets have been observed in the parenchymal cells than in the Kupffer cells [107]

The tissues capable of removing chylomicrons include liver heart skeletal muscle and adipose tissue [108] In vivo studies [108] suggest but have not proved that adequate carbohydrate metabolism is necessary for uptake by adipose tissue The isolated perfused liver has been shown to be capable of both removing and oxidizing chylomicron triglyceride fatty acids (TGFA) [107] and it is probable that this is true of most other sites of removal Many of the chylomicron triglyceride fatty acids are oxidized shortly after removal and it is well established that these incoming fatty acids are readily available for metabolism [57]

There are indications that chylomicrons are degraded during their removal As chylomicrons leave the blood the triglyceride fatty acids rapidly reappear in the blood as UFA [109] It has been estimated that much of the TGFA is directly oxidized without retransport as UFA [34] but hydrolysis may still be a necessary step in the tissue uptake of chylomicron triglyceride

Granted the close association between hydrolysis and removal of chylomicrons there are two schools of thought concerning the site of hydrolysis and participation of other plasma lipoproteins in this process

Observations of labeled chylomicron removal in the rat [111] and the dog [109] suggest that chylomicron triglyceride does not pass through other low-density lipoproteins in plasma during its removal or if it does that these other lipoproteins turn over with extremely rapid rates This implies that at tissue cell interfaces either chylomicrons are removed intact or the triglyceride is hydrolyzed with removal of some or all of the hydrolysis products by the cells

The possibility that chylomicrons may escape the vascular system intact is supported by the observations that their removal from the blood is accompanied by the appearance of a small concentration of chylomicrons in the hepatic lymph [112] Capillary pores in the endothelial wall have been observed by electron microscopy [113] they suggest a *possible means of egress*

On the other hand it has been suggested that particularly in man the degradation of chylomicrons takes place primarily intravascularly This involves the hydrolysis of triglyceride with the removal of the product fatty acids as UFA bound to albumin leaving residual lipoproteins of higher density which may be degraded sequentially This theory has

## POSSIBLE BIOCHEMICAL DEFECTS IN ESSENTIAL HYPERLIPEMIA

It must be emphasized again that very probably not all cases currently defined as essential hyperlipemia arise from the same metabolic defect In no case is the exact mechanism understood Two possible sources for the excessive amounts of glyceride in the plasma exist (1) defective removal from blood of exogenous glyceride absorbed from the diet (2) release into the plasma of more endogenous glyceride than can be removed at a rate sufficient to maintain normal concentrations These two possibilities may be considered in turn approached first from the standpoint of what is known of the normal mechanisms involved

*Removal of Exogenous Triglyceride*

**Absorption and Chylomicron Formation** The fat in the diet consists largely of triglyceride of which 50 to 100 gm is consumed daily by the average adult The mechanisms of digestion of fat and its transfer across the intestinal mucosal cell into the lymph are only partially understood For detailed analysis of current research in this area reference may be made to recent reviews by Bergstrom and Borgstrom [103] and Frazer [104]

From work of Bloom et al [105] and Bergstrom and Borgstrom and their colleagues [103] it is known that the fatty acids of chain length $C_{10}$ and greater are reincorporated into triglycerides during absorption and transported to the blood in the lymph via the thoracic duct Small amounts of some acids such as stearic are also transported as phospholipid Very little is transported as unesterified fatty acid or as cholesterol esters

As the glycerides of acids of longer chain length are re formed in or near the mucosal cells they appear in the intestinal lacteals as chylomicrons As the chylomicrons accumulate the lymph becomes lactescent and the milky chyle is carried to the blood via the thoracic duct The maximum concentration of glyceride and chylomicrons is usually reached 3 to 4 hr after ingestion of fat Increased amounts of cholesterol and phospholipid are also carried in lymph with the chylomicrons and their concentrations in plasma may rise during fat absorption After the chylomicrons have disappeared small increases in lipid in higher density lipoproteins may remain for short periods

**Chylomicron Removal** The mechanisms for removing chylomicrons from the blood are only partially understood It is not known whether the chylomicrons pass into tissues intact or whether they must first be degraded either at the capillary cell interface or in the blood It is known that the triglyceride moiety of chylomicrons is rapidly removed from the blood at an exponential rate and that the fractional turnover rate (the fraction of the total amount in blood disappearing per unit time) is

**Factors Influencing Chylomicron Removal** One of the more important factors affecting chylomicron removal appears to be carbohydrate metabolism Retardation in removal of fat emulsions from the plasma of diabetic dogs has been demon trated [*122*] Recent experiments in human beings have been interpreted as indicating that alimentary lipemia is decreased by factors promoting carbohydrate utilization such as glucose feeding or glucagon administration and increased by factors decrea ing carbohydrate utilization such as fasting or epinephrine administration [*123*] Carbohydrate feeding has not yet been shown experimentally to increase chylomicron removal and the effect of carbohydrate metabolism on fat absorption has not been adequately explored as a possible explanation of the above phenomena

**Possible Defects in Chylomicron Metabolism in Essential Hyper lipemia** The concept that in essential hyperlipemia exogenous fat is removed at an abnormally slow rate (so-called retention hyperlipemia [*21*]) has been supported by some pertinent observations These include the well established clinical experience that hyperlipemia in such patients is often a direct function of dietary intake of fat An intake over 30 to 50 gm per day will lead to marked hyperlipemia and in a case such as that of Holt et al [*71*] to abdominal crises and marked hepatic and splenic enlargement This latter suggests that removal of chylomicrons by reticuloendothelial phagocytosis becomes significant in extreme degrees of hyperlipemia A low fat intake diet will always reduce the hyper lipemia In hyperlipemic members of the P family (see below) fat free diets restore blood lipids to very nearly normal levels in a few days Havel [*124*] injected intravenously lipemic plasma from one of these patients into the patient after his blood lipids had been normalized by diet and into a normal control In the patient the chylomicrons dis appeared over a matter of days in the normal person they were removed in a few hours

A slower rate of clearance from the blood of fed $I^{131}$ triolein [*125 126*] or intravenously injected olive oil or triolein emulsions [*127*] has been reported in patient with essential hyperlipemia when compared with normal persons or patients with essential hypercholesterolemia without hyperlipemia Since presence of hyperlipemia may overload the chylomi cron clearing mechanism and decrease the fractional removal rate of chylo-microns experiments designed to prove a basic defect in clearing should properly be conducted with the recipient in a normolipemic state Only a few experiments such as those reported by Havel [*124*] and by Crofford et al [*126*] provide unequivocal evidence of delayed chylomicron removal in es ential hyperlipemia

Enough evidence is available to permit peculation about only three possible sources of the defect in chylomicron metabolism The e are a deficiency in lipoprotein lipase activity pre ence of abnormal lipoproteins

arisen largely from the work of Gofman and his colleagues who have shown that during the clearing of hyperlipemia there occur stepwise increases in low density lipoproteins of progressively greater density and lower flotation rates [78] This "interconversion" has also been reported in human beings following the ingestion of a fat meal [114] There is no doubt that "interconversion" within certain low density lipoprotein classes may occur physiologically a defect in such "conversion" has been proposed as the origin of hyperlipemia in the nephrotic syndrome [59]

Although experiments employing labeled phospholipid [115] cholesterol [57] or protein [66] portions of the chylomicrons have shown that these moieties leave the plasma more slowly than the triglyceride portion it has not been possible to determine whether lipoprotein "skeletons" of higher density are actually left in the blood after departure of the chylomicron triglyceride This is because both labeled protein and lipid may passively exchange between chylomicrons and other lipoproteins a process which may mimic actual metabolic "conversion" Thus a defect in "conversion" of chylomicrons in essential hyperlipemia remains hypothetical

**Lipoprotein Lipase** It is obvious from the foregoing discussion that some hydrolytic enzyme system or systems plays a pivotal role in chylomicron metabolism and no doubt in the metabolism of endogenous triglyceride and UFA While several lipases have been isolated from adipose tissue blood and the pancreatic secretion the lipase considered most seriously to have a role in fatty acid transport is lipoprotein lipase [116] It catalyzes the hydrolysis of triglycerides in any plasma lipoprotein or in synthetic emulsions provided a small amount of lipoprotein is available for activation [116] When the reaction occurs in plasma the UFA released by the reaction are bound by albumin Di and monoglycerides are also formed [80] The degradation of the triglycerides is accompanied by fall in the concentration of the larger light-scattering lipoproteins (which are especially rich in glyceride) and the plasma becomes less turbid Hence the original designation of the enzymatic activity as clearing factor Under physiologic conditions very little lipoprotein lipase activity is measurable in plasma in man [117] or the rat [118] The activity is increased somewhat by fat feeding From in vitro measurements of activity in human plasma it has been estimated that the hydrolysis taking place would be sufficient to account for only a small part of the hydrolysis necessary to clear normal fat loads Plasma lipoprotein lipase activity is increased manyfold by the administration of heparin or heparin like substances The mechanism of this activation or leakage of enzyme activity into the plasma is not known It is of interest however that bacterial heparinase can destroy the activity of lipoprotein lipase [119] Lipoprotein lipase has been isolated from rat heart and adipose tissue [116 120] but so far not from liver In fact the latter organ appears to remove lipoprotein lipase activity from plasma [121]

Theoretically any factor interfering with the processes involved in UFA and fatty acid ester metabolism within the cell (see below) or plasma UFA transport may also interfere with chylomicron removal The author has measured plasma UFA turnover and oxidation of plasma UFA by isotopic means [129] in four nonfamilial cases of essential hyperlipemia without detection of variation from the still crudely defined limits of normal

**Abnormal Lipoproteins** It has recently been suggested by Schettler et al [85] that chylomicrons in essential hyperlipemic patients may have abnormal chemical composition Their experimental data however are not based on well washed chylomicrons free of possible contamination with other lipoproteins Plasma chylomicron from the author s patient J L (Table 16–4) have concentrations of cholesterol pho pholipid and glyceride similar to the concentration of chylomicrons isolated from pooled normal plasma The N terminal amino acid residues of the chylomicron protein were also similar to lymph chylomicrons obtained from a normolipemic patient It is to be noted that chylomicrons of the P family members are readily cleared by normal per ons

Abnormally slow hydrolysis of chylomicrons from patients with essential hyperlipemia upon in vitro incubation with lipoprotein lipase has been reported [29 130] In the second of the e cases Carlson and Olhagen [130] obtained a reduction in plasma glycerides after heparin administration Their carefully studied patient had several features suggestive of essential familial hypercholesterolemia especially no fall in a markedly elevated cholesterol concentration after 4 days of fasting They concluded that the defect in this patient may have been primarily in endogenous glyceride metabolism

It has already been indicated that decreases in some higher density lipoproteins occur in hyperlipidemia especially in marked chylomicronemia The lack of specificity of the e changes and frequent reversion toward normal as the hyperlipemia is reduced suggest that this is a secondary phenomenon It has been suggested that apparent deficiencies of lipoproteins of density > 1 019 in hyperlipemia may be due to a leaching of their lipid by the excessive concentration of chylomicrons [85 131] A primary deficiency in high density lipoprotein could theoretically deprive incoming chylomicrons of sufficient protein for stabilization and activation for lipolysis Observation that clearing of hyperlipemia in patients with essential familial hyperlipemia may still not correct abnormally low concentrations of high den ity lipoprotein [124] leaves this intriguing problem yet to be solved

## ENDOGENOUS GLYCERIDE METABOLISM AND HYPERLIPEMIA

With the possible exception of the unu ual P family it has not been shown that the hyperglyceridemia in e sential familial hyperlipemia

or abnormal carbohydrate metabolism secondarily affecting the metabolism of glyceride It will be more convenient to consider the last possibility later with a discussion of endogenous glyceride metabolism

**Lipoprotein Lipase Deficiency** Reference has already been made to the discovery by Havel [*124*] of three hyperlipemic siblings whose plasma contains abnormally low lipoprotein lipase activity after injection of up to 100 mg heparin intravenously (the P family) Enzyme activity is negligible as measured by changes in optical density or in UFA or glycerol concentrations when their postheparin plasma is incubated with their own chylomicrons or artificial triglyceride emulsions Enzyme activity is present after much smaller doses of heparin in the one other familial case the author has studied (J L, Table 16-4) and in other cases of essential hyperlipemia' in which examination for presence of this enzyme in *plasma has been reported A possible exception is the brief mention* by Crofford et al [*126*] of an abnormal heparin response in a hyperlipemic patient

The paucity of enzyme in the plasma of the P family can only be assumed to represent a *tissue deficiency* interfering with *hydrolysis and* delaying chylomicron removal Since activity of this enzyme has not yet been measured in human tissue it has not been possible to test for deficiency in the hyperlipemic members It is very probable however that this family represents a rare subgroup of essential familial hyperlipemia and possibly a unique mutation It is interesting that these siblings (Fig 16 5) who refuse to restrict their fat intake, have had thirty to fiftyfold elevations in glyceride concentrations (Table 16-4) for at least 11 years and probably since birth One had a 3 day bout of abdominal pain with transient splenomegaly for the first time at age 23 None has any evidence of atherosclerosis

Standardized measurements of plasma lipoprotein lipase activity following heparin have not been made in a comparison study of essential familial hyperlipemic patients and normal persons matched for age and sex It is essential that such studies employ UFA or glycerol production rather than optical clearing for measurement of activity A *relative* deficiency in enzyme response to heparin has therefore not been excluded in other patients with this syndrome Evidence has already been presented indicating that if lipoprotein lipase has a physiologic role in chylomicron removal the activity of the enzyme in tissues is most likely more important than in plasma Adequate assay of lipoprotein lipase activity or of heparin or its analogues has not been made in human tissues

No inhibitor of lipoprotein lipase activity is present in the plasma of the P family [*124*] Lever and Klein [*128*] have reported inhibition of the enzyme in vitro following addition of hyperlipemic plasma obtained from patients with essential hyperlipemia or von Gierke s disease Their *results suggest a nonspecific factor* which needs to be further investigated

have been reported abnormal in a series of patients with "essential" hyperlipemia or hypercholesterolemia [132] Recent interesting observations indicate that hyperglycemia may depend upon the degree of hyperlipemia [126]

Most patients with unequivocal essential familial hyperlipemia have had a normal glucose tolerance Thannhauser has emphasized that insulin did not affect the blood lipids in his patients with glycosuria [21] This was likewise true for patient A S in Table 16 5 The pathways for metabolism of fat and carbohydrate are so intertwined however that it is not surprising to find abnormalities in both in hyperlipidemia What is known of these interrelationships offers a few furtive clues to the causes of essential hyperlipidemia

SYNTHESIS OF GLYCERIDES AND GLYCEROPHOSPHATIDES

The scheme presented in Fig 16-4 is that suggested by Kennedy [133] who with his coworkers is primarily responsible for discovery of the essential role of the cytidine phosphatides as essential cofactors in these pathways It is apparent that once the common intermediate 1 2 diglyceride has been formed the reactions may proceed to the synthesis of either triglyceride or glycerophosphatide This relationship explains some observations of increased phospholipid turnover at times when the major process is net synthesis of triglyceride

Although it is assumed that lipoprotein lipase activity in adipose tissue may be the physiologic mechanism for releasing UFA it is unlikely as Kennedy has pointed out [133] that reversal of lipolysis a low energy reaction accounts for much net synthesis of adipose tissue triglycerides This is true despite the finding that labeled acids are incorporated into glycerides in simple equilibrium experiments catalyzed by lipoprotein lipase [134]

The net synthesis of triglycerides involves the intermediate formation of phosphatidic acid and 1 2 diglyceride ATP and acyl CoA are essential for these steps [133] as is available glycerol and it is logical to assume that a major share of the latter comes from glucose degradation The major role of carbohydrate metabolism in supplying both parts and energy for glyceride and glycerophosphatide synthesis is an important example of interdependence of carbohydrate and lipid metabolism

In any tissue the metabolism of triglycerides must also be intimately related to the metabolism of fatty acids themselves Recent reviews of progress in this area include those of Lehninger [135] and Langdon [136] Probably the enzymes necessary for synthesis of glycerides exist in all tissues capable of synthesizing fatty acids Two of the most active in this capacity are the liver and the adipose tissue Shapiro [137] has recently reviewed the evidence indicating that at least a large part of the adipose tissue triglycerides is synthesized *in situ* Fatty acid synthesis

depends entirely upon exogenous fat intake Extremely low fat diets have rarely been maintained long enough to eliminate endogenous glyceride as a source of hyperlipemia

The author has observed at least six patients with rather severe "essential hyperlipemia which is not obviously dependent upon fat intake This is indicated in Table 16-5 where results of feeding isocaloric formula

TABLE 16-5 THE EFFECTS OF VARYING FAT INTAKE AND INSULIN IN ATYPICAL ESSENTIAL HYPERLIPEMIA

| Patient | Day | Daily fat intake | TG mg/100 ml | TC mg/100 ml | FC mg/100 ml | PL mg/100 ml |
|---|---|---|---|---|---|---|
| N C (age 64) | -[illegible]0 | c 100 gm | 8 100 | 1 118 | 4[illegible]4 | 1 242 |
| | 0 | Begin 5 gm | 3 [illegible]26 | 610 | 210 | [illegible]0 |
| | 21 | Begin 100 gm (corn oil) | 76[illegible] | 176 | 68 | 281 |
| | 28 | Begin 5 gm | 817 | 165 | 64 | [illegible]6[illegible] |
| | 36 | Begin 100 gm (coconut oil) | 601 | 163 | 6[illegible] | [illegible]51 |
| | 42 | Begin 100 gm (butter fat) | 5[illegible]8 | 172 | 63 | [illegible]58 |
| | 48 | Begin 5 gm | 8[illegible]5 | 229 | 85 | 334 |
| | 62 | End 5 gm | 255 | 136 | 36 | 195 |
| A S (age 40) | 0 | Begin 5 gm | 1 034 | 353 | 118 | 404 |
| | 11 | Begin 100 gm (corn oil) | 1 04[illegible] | 28[illegible] | 103 | 381 |
| | 21 | End 100 gm | 453 | 248 | 60 | 261 |
| | 0 | Regular insulin 15 units twice daily | 523 | 314 | 80 | 340 |
| | 7 | End regular insulin 15 units twice daily | 6[illegible]5 | 363 | 103 | 3[illegible] |

NOTE TG = triglyceride TC = total cholesterol FC = free cholesterol PL = phospholipid Day O and O indicate the start of specific experimental periods

diets to two such patients are presented Both these patients were obese adult males who also had occasional glycosuria fasting blood sugar levels of 100 to 120 mg per 100 ml and moderately abnormal glucose tolerance test results A family history of hyperlipidemia was not elicited but no relatives were available for analyses Subsequent reduction to ideal body weight has left both patients with nearly normal glucose tolerance and glyceride concentrations not over 250 mg per 100 ml unaffected by isocaloric fat intake

These cases are consistent with hyperlipemia of endogenous origin related to abnormal carbohydrate metabolism The association of reasonably controlled diabetes with persistent hyperlipemia has been reported in a number of patients [89] Glucose and insulin tolerance test results

concentrations occur when intake of food not necessarily fats exceeds caloric demand These hyperlipemias of feast or of famine have not been shown experimentally to involve increased *net* transport of fatty acids as glycerides but they probably do They must both be interpreted in the light of what is known about the capacity for transport of fatty acids in plasma as UFA This transport system with its complementary relationship to carbohydrate metabolism is also linked inseparably to glyceride metabolism

When carbohydrate utilization is diminished below some critical level UFA pours into the plasma and is taken up by tissues like the liver and muscle to fill the need for oxidizable substrate As measured by isotopic means this turnover of plasma UFA may be even greater than the caloric demand [*41* *129*] It is conceivable that excess UFA may be converted to glyceride which spills back into the plasma It is not excluded of course that fatty acids may leave the depots directly as glyceride perhaps in response to some stimulus other than need for substrate and merely pile up in plasma having exceeded the capacity of other tissue to remove them There is good evidence that whatever the form in which they are initially released the elevated fatty acids have their origin in adipose tissue [*41*] Adipose tissue may also be reluctant to take them back in the absence of adequate carbohydrate utilization [*108*]

When carbohydrate utilization is normal and supply exceeds demand some of the excess may be converted to glyceride Hyperlipemia may result from the transport of this glyceride to adipose tissues for storage That adipose tissue is capable of synthesizing its own glycerides from carbohydrate [*137*] does not weaken this hypothesis This hyperlipemia of caloric excess would of course be exaggerated in patients with any defect in chylomicron metabolism

*Humoral Factors*

Several humoral factors have been reported which may cause fat mobilization and sometimes hyperlipemia These include factors isolated from the anterior pituitary [*138* *139*] from the blood of cortisone treated animals or extracts of the posterior pituitary [*140*] or from the urine of fasting human beings [*141*] Epinephrine causes hyperlipemia in certain species but the relationship of this action to effects of epinephrine on the adrenal cortex and on carbohydrate utilization is yet to be determined Glucocorticoids have been shown to be necessary for production of hyperlipemia in diabetic rats deprived of insulin [*142*] The hyperlipemia coincides with the development of hyperglycemia and ketosis The relationship of emotional stress to hyperlipemia has been little explored although transient elevation of lipid predominantly cholesterol in patients under emotional strain has been reported [*143*]

None of the general understanding of the interrelationships of car

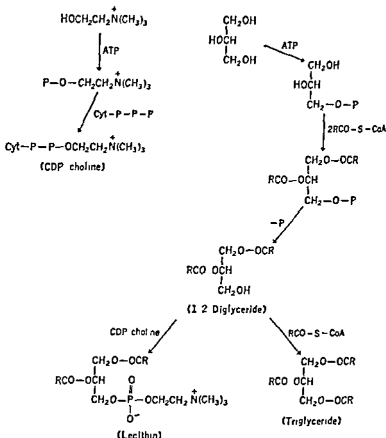



Fig 16-4 The relationship between phospholipid and triglyceride synthesis (after Kennedy [133]) illustrating the pivotal position of 1 2 diglycerides The reactions for formation of CDP choline (cytidine diphosphate choline) and lecithin have been shown to be identical for the formation of CDP ethanolamine and cephalin (phosphatidyl ethanolamine)

is dependent upon carbohydrate metabolism as a source of adequate TPNH [136]

## REGULATION OF ENDOGENOUS TRIGLYCERIDE CONCENTRATIONS IN PLASMA

### *Carbohydrate Metabolism*

In the absence of fat ingestion the glyceride concentrations are not negligible being of the order of 100 mg per 100 ml after 16 hr of fast With continued fasting triglyceride concentrations do not tend to diminish but are on the contrary often increased The principal lipoproteins which may be elevated include chylomicrons and it is assumed they are formed at sites other than the intestine Conversely increased glyceride

concentrations occur when intake of food not necessarily fats exceeds caloric demand These hyperlipemias of feast or of famine have not been shown experimentally to involve increased *net* transport of fatty acids as glycerides but they probably do They must both be interpreted in the light of what is known about the capacity for transport of fatty acids in plasma as UFA This transport system with its complementary relationship to carbohydrate metabolism is also linked inseparably to glyceride metabolism

When carbohydrate utilization is diminished below some critical level UFA pours into the plasma and is taken up by tissues like the liver and muscle to fill the need for oxidizable substrate As measured by isotopic means this turnover of plasma UFA may be even greater than the caloric demand [*41 129*] It is conceivable that excess UFA may be converted to glyceride which spills back into the plasma It is not excluded of course that fatty acids may leave the depots directly as glyceride perhaps in response to some stimulus other than need for substrate and merely pile up in plasma having exceeded the capacity of other tissue to remove them There is good evidence that whatever the form in which they are initially released the elevated fatty acids have their origin in adipose tissue [*41*] Adipose tissue may also be reluctant to take them back in the absence of adequate carbohydrate utilization [*108*]

When carbohydrate utilization is normal and supply exceeds demand some of the excess may be converted to glyceride Hyperlipemia may result from the transport of this glyceride to adipose tissues for storage That adipose tissue is capable of synthesizing its own glycerides from carbohydrate [*137*] does not weaken this hypothesis This hyperlipemia of caloric excess would of course be exaggerated in patients with any defect in chylomicron metabolism

*Humoral Factors*

Several humoral factors have been reported which may cause fat mobilization and sometimes hyperlipemia These include factors isolated from the anterior pituitary [*138 139*] from the blood of cortisone-treated animals or extracts of the posterior pituitary [*140*] or from the urine of fasting human beings [*141*] Epinephrine causes hyperlipemia in certain species but the relationship of this action to effects of epinephrine on the adrenal cortex and on carbohydrate utilization is yet to be determined Glucocorticoids have been shown to be necessary for production of *hyperlipemia in diabetic rats deprived of insulin [142]* The hyperlipemia coincides with the development of hyperglycemia and ketosis The relationship of emotional stress to hyperlipemia has been little explored although transient elevation of lipid predominantly cholesterol in patients under emotional strain has been reported [*143*]

None of the general understanding of the interrelationships of car

bohydrate and fat metabolism nor any of these isolated data on humoral factors, has yet been translated into specific mechanisms regulating either endogenous or exogenous triglyceride metabolism Until this has been accomplished for the normal state any defective enzyme systems which may lie at the root of essential hyperlipemia will not be understood

## GENETICS

### *Mode of Inheritance*

Adequate data are not available to ascertain the mode of inheritance of essential familial hyperlipemia Not only have relatives often not been examined but reports of blood lipid findings have been inadequate It cannot always be ascertained whether the hyperlipidemia seen in affected relatives is even of the same type as that in the propositus

It has been stated by Hirschhorn et al [91] that familial hyperlipemia" is a dominant trait in which the homozygous phenotype includes hepatosplenomegaly, abdominal pain and other features shown in Table 16-3 and the heterozygous a tendency toward hyperlipidemia and atherosclerosis This inference is derived by the authors from the single case report of Holt and coworkers [71] While this supposition may prove ultimately to be correct it is not yet supported by sufficient case analyses and is rendered especially tenuous by the present difficulty in defining the trait or traits involved

Of the cases elected here for presentation as essential familial hyperlipemia 10 pedigrees were known to have been affected more than once One parent of the propositus was also involved in three instances [71, 74, 77] In one of these a maternal aunt in addition to the mother was said to have had elevated blood lipids In the remaining 7 pedigrees affected individuals have been reported only in a sibship but the parents were specifically stated or known to have been examined and found to be normal in only 2 of these pedigrees

The pedigree of the Negro P family is shown in Fig 16-5 The mother and father have slightly elevated total cholesterol concentrations about 200 mg per 100 ml but clear serums with no remarkable elevation in triglycerides have been observed in recent years Five years earlier Gaskins [79] had found the mother to have a triglyceride concentration of 1 600 mg per 100 ml Neither parent has xanthomas or hepatosplenomegaly and there is no traceable consanguinity in the marriage The unaffected siblings have completely normal blood lipid levels Havel [124] found that only the 3 hyperlipemic members had an abnormal response to heparin Lack of reproducible abnormality in either parent suggests that this is not a simple dominant trait and that it could be a completely penetrant recessive trait in this instance It is of interest in regard to gene locus that only the hyperlipemic siblings also have sickle cell trait

with abnormal amounts of hemoglobin S Franklin's [144] report of a single case in a 5 year old in which the parents and 4 other siblings were normal also indicates that the disease can be recessive Lipoprotein lipase was unknown at that time and comparison with the P family is not meaningful There is an apparent predilection for males (Table 16-3) but further supporting data are needed before a sex controlled factor may be considered

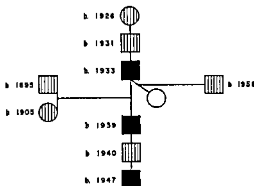



Fig 16-5 Pedigree of the P family representing a variant of essential familial hyperlipemia The three hyperlipemic siblings have deficient lipoprotein lipase activity in plasma after heparin administration Elevated triglycerides were obtained in the mother (born 1905) on one occasion [7J] but not 5 years later She is classified here as normal

The syndrome has appeared in both the white and Negro races and there is no demonstrated preponderance in any ethnic group

*Definition of the Hyperlipemic Trait*

There is need for both more uniformity and more ingenuity in defining the trait or traits responsible for essential hyperlipemia The minimum study of such patients should include (1) chemical determination of at least plasma cholesterol phospholipids and glycerides with utilization of UFA and lipoprotein analyses as well as further methods of lipid fractionation if available (2) determination of plasma lipoprotein lipase activity a few minutes after injection of 10 mg sodium heparin intravenously as determined by UFA production or glyceride disappearance in addition to optical clearing (3) a glucose tolerance test after adequate preparation by carbohydrate feeding (4) thorough screening of relatives by the same tests especially complete plasma lipid analyses (5) response of the plasma lipids both to reduction to ideal weight if the patient is obese and to diets containing variable amounts of fat

Upon this base more specific methods of trait definition can be developed and the way opened to understanding of this most intriguing phenomenon

bohydrate and fat metabolism nor any of these isolated data on humoral factors has yet been translated into specific mechanism regulating either endogenous or exogenous triglyceride metabolism. Until this has been accomplished for the normal state any defective enzyme system which may lie at the root of essential hyperlipemia will not be understood.

GENETICS

### *Mode of Inheritance*

Adequate data are not available to ascertain the mode of inheritance of essential familial hyperlipemia Not only have relatives often not been examined but reports of blood lipid findings have been inadequate It cannot always be ascertained whether the hyperlipidemia seen in affected relatives is even of the same type as that in the propositu

It has been stated by Hirschhorn et al [91] that familial hyperlipemia is a dominant trait in which the homozygous phenotype includes hepatosplenomegaly abdominal pain and other features shown in Table 16-3 and the heterozygous a tendency toward hyperlipidemia and atherosclerosis This inference is derived by the authors from the single case report of Holt and coworkers [71] While this supposition may prove ultimately to be correct it is not yet supported by sufficient case analyses and is rendered especially tenuous by the present difficulty in defining the trait or traits involved.

Of the cases selected here for presentation as essential familial hyperlipemia 10 pedigrees were known to have been affected more than once. One parent of the propositus was also involved in three instances [71 74 77] In one of these a maternal aunt in addition to the mother was said to have had elevated blood lipids. In the remaining 7 pedigrees affected individuals have been reported only in a sibship but the parent were specifically stated or known to have been examined and found to be normal in only 2 of these pedigrees.

The pedigree of the Negro P family is shown in Fig 16-5 The mother and father have slightly elevated total cholesterol concentrations about 200 mg per 100 ml but clear serums with no remarkable elevation in triglycerides have been observed in recent years Five years earlier Gaskins [79] had found the mother to have a triglyceride concentration of 1 600 mg per 100 ml Neither parent has xanthomas or hepatosplenomegaly and there is no traceable consanguinity in the marriage The unaffected siblings have completely normal blood lipid levels. Havel [124] found that only the 3 hyperlipemic members had an abnormal response to heparin Lack of reproducible abnormality in either parent suggest that this is not a simple dominant trait and that it could be a completely penetrant recessive trait in this instance It is of interest in regard to gene locu that only the hyperlipemic siblings also have sickle-cell trait

with abnormal amounts of hemoglobin S Franklin's [144] report of a single case in a 5-year-old in which the parents and 4 other siblings were normal also indicates that the disease can be recessive Lipoprotein lipase was unknown at that time and comparison with the P family is not meaningful There is an apparent predilection for males (Table 16-3) but further supporting data are needed before a sex-controlled factor may be considered

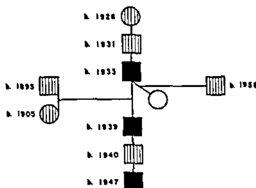



Fig 16-5 Pedigree of the P family representing a variant of essential familial hyperlipemia The three hyperlipemic siblings have deficient lipoprotein lipase activity in plasma after heparin administration Elevated triglycerides were obtained in the mother (born 1905) on one occasion [73] but not 5 years later She is classified here as normal

The syndrome has appeared in both the white and Negro races and there is no demonstrated preponderance in any ethnic group

*Definition of the Hyperlipemic Trait*

There is need for both more uniformity and more ingenuity in defining the trait or traits responsible for essential hyperlipemia The minimum study of such patients should include (1) chemical determination of at least plasma cholesterol phospholipids and glycerides with utilization of UFA and lipoprotein analyses as well as further methods of lipid fractionation if available (2) determination of plasma lipoprotein lipase activity a few minutes after injection of 10 mg sodium heparin intravenously as determined by UFA production or glyceride disappearance in addition to optical clearing (3) a glucose tolerance test after adequate preparation by carbohydrate feeding (4) thorough screening of relatives by the same tests especially complete plasma lipid analyses (5) response of the plasma lipids both to reduction to ideal weight if the patient is obese and to diets containing variable amounts of fat

Upon this base more specific methods of trait definition can be developed and the way opened to understanding of this most intriguing phenomenon

## COURSE

As defined here essential familial hyperlipemia must be considered a relatively benign condition Deaths have been reported in three cases One of these [96 145] was almost certainly due to incidental causes Two of the three brothers mentioned by Harslof [75] died rather suddenly in their youth without a known cause In the forty some cases reviewed clinical evidence of atherosclerosis has not been present This factor however entered into the exclusion from this series of the "mixed cases [90] or possible hyperlipemic variants of essential familial hypercholesterolemia In these latter cases accelerated development of atherosclerosis is an outstanding complication, this must be considered potentially true for all cases of essential hyperlipemia Postprandial hyperlipemia has been shown to be associated with development of electrocardiographic signs of myocardial insufficiency [146] decreased coronary blood flow [147] and inhibition of fibrinolysis [148] as well as with acceleration of blood clotting [149] For these reason as well as the desirability of avoiding abdominal pain pancreatitis and eruptive xanthomas and foam cell development in various organs effort should be made to decrease the constant hyperlipemia

## TREATMENT

The restriction of total fat in the diet to the lowest practical and palatable minimum (30 to 50 gm per day) is the most effective therapy for essential familial hyperlipemia at present In some cases of both essential hyperlipemia and the hyperlipemia associated with essential hypercholesterolemia accompanied by obesity reduction to ideal weight may produce a dramatic decrease in plasma glycerides Spaced fat feeding has also been recommended to decrease hyperlipemia [152]

There is evidence that diets extremely low in fat and necessarily high in carbohydrate may cause the low density lipoproteins of high flotation rate and consequently the glyceride concentration to rise [150 151] This does not appear to be true for some essential hyperlipemic patients maintained on 5 gm fat diets for short periods (Table 16 5) but the long term effect of such extreme diets has not been explored It is also not known at present whether different kinds of dietary fat i e saturated or unsaturated fats have different effects on triglyceride levels in essential familial hyperlipemia

Heparin has been used in therapeutic trials by continuous intravenous infusion or repeated subcutaneous injection [153] All the patients so treated by Lever Herbst and Hurley [153] responded with some clearing of the hyperlipemia In none were glycerides reduced to normal levels and there were levels of lipemia below which heparin seemed to be less effective Most significantly no long standing remission was induced by a

single course of heparin To be beneficial heparin would no doubt have to be used as a chronic form of therapy For this purpose parenteral heparin is quite unsatisfactory even apart from its potential hazard as an anticlotting agent

### SUMMARY

1 Essential familial hyperlipemia is a syndrome characterized by hyperglyceridemia and elevated concentrations of chylomicrons and low density lipoproteins in the blood The hyperlipemia is directly related to the intake of fat in the diet It is often accompanied by eruptive xanthomas hepatosplenomegaly and abdominal pain Rarely there may be associated febrile crises or pancreatitis

2 The familial occurrence of the syndrome does not of itself define a distinct trait and more than one biochemical lesion may no doubt give rise to similar clinical features Somewhat arbitrary grounds have been used here to distinguish the syndrome from other types of hyperlipidemia such as that associated with essential familial hypercholesterolemia

3 The cause (or causes) is unknown but appears to be related mainly to defective removal of chylomicrons from the plasma Defective metabolism of endogenous glyceride may also be involved One possibly unique pedigree has been found the hyperlipemic members of which appear deficient in lipoprotein lipase activity This enzyme may have wide significance in triglyceride and fatty acid metabolism

4 Males appear to be predominantly affected but the lack of a clearly definable trait and scarcity of data do not permit proposal of any genetic model

## ESSENTIAL FAMILIAL HYPERCHOLESTEROLEMIA

### HISTORICAL ASPECTS

The concept of an inheritable tendency toward development of hypercholesterolemia frequently associated with xanthomatosis and atherosclerosis has evolved slowly from the first pertinent observations made over 100 years ago

Cutaneotendinous xanthomas were first reported in 1836 by Rayer [154] and in 1851 by Addison and Gull [155] Occurrence of these lesions in siblings was soon observed by a number of clinicians [156–159] and at the same time it became obvious that they might be accompanied by severe xanthomatous involvement of the heart and great vessels [158 160–162] In 1910 Arning [163] reported xanthomatosis in a mother and four of her nine children at least one of whom died suddenly in childhood By 1930 the syndrome of familial xanthoma [159] or hereditary xanthoma tuberosum multiplex was well established more cases than can be accurately compiled have been subsequently observed

Quinquad (1873) has been credited [164] with first suggesting that

xanthoma development was related to the blood lipids The studies of xanthoma cells by Pick and Pinkus beginning in 1908 [165] provided the first support for such an hypothesis Chauffard and LaRoche [164] observed as early as 1910 that hypercholesterolemia accompanied xanthomas in patients with obstructive liver disease They concluded that " what the tophus is to gout the xanthoma is to hypercholesterolemia ' Pollitzer and Wile [166] in 1912 expressed a similar opinion after histologic studies of the serial development of xanthoma tuberosum It was not until 1920, however that hypercholesterolemia was actually demonstrated in a patient with xanthomatosis without obstructive liver disease [167]

Separation of essential hypercholesterolemia as a syndrome different from other diseases sometimes associated with xanthomas has evolved gradually as classification of xanthomatoses has improved [168–171] The contributions of Thannhauser and Magendantz [171] and Thannhauser [21] have been especially helpful in this regard Thannhauser prefers the term *familial hypercholesterolemic xanthomatosis* separating as a *forme fruste* those patients with essential hypercholesterolemia without xanthomatosis [21] This terminology is somewhat distracting since it is apparent that xanthomatosis is a secondary manifestation dependent upon duration of hypercholesterolemia [172] and possibly genotype [173]

For this reason the name *essential familial hypercholesterolemia* suggested by Wilkinson, Hand and Fliegelman [173] seems more appropriate These authors published the first large-scale studies of involved families and numerous similar studies have been subsequently reported (see below) As a result of these the genetics of hypercholesterolemia are partially understood and the clinical manifestations well known

The frequency with which hypercholesterolemia and atherosclerosis are encountered today however tends to blur the image of essential familial hypercholesterolemia and to complicate the separation of inherited and acquired factors producing hyperlipidemia As a substitute for cholesterol measurements lipoprotein analyses have been employed by a number of workers especially Gofman and his colleagues [1] Significant correlations between concentrations of low density lipoprotein classes and atherosclerosis [174] or various xanthomas [175] have been demonstrated The separation of specific defects in lipoprotein transport as inheritable traits has not yet been accomplished and the biochemical bases for either the hyperlipidemia or hyperlipoproteinemia remain obscure

## CLINICAL ASPECTS

### *Definition and Manifestations*

Essential familial hypercholesterolemia is a syndrome characterized by abnormally high concentrations of cholesterol and phospholipid and

sometimes of glycerides in the blood Apparently depending upon the severity and duration [172] of the hyperlipidemia and also upon the nature of the abnormal lipid and lipoprotein pattern [175] this syndrome is often accompanied by cutaneous and tendon xanthomas (Figs 16 6 to 16-9) corneal arcus and most seriously a tendency toward accelerated development of atherosclerosis The syndrome is definitely familial and may involve either sex It may manifest itself in the first years of life with severe xanthomatous involvement of the skin endocardium heart valves coronary arteries and other organs ending in sudden death from



Fig 16-6 Tendon xanthomas on the fingers

myocardial infarction in childhood or it may be present as nothing more than a slight elevation in plasma cholesterol level with no limitation of the normal life span

**Diagnosis** The diagnosis of essential familial hypercholesterolemia depends upon the demonstration of a cholesterol level which is abnormally high for the age sex and population group with which the patient is compared The establishment of a cutoff point between normal and abnormal is arbitrary and varies with each laboratory For laboratories using accepted methods well standardized 300 mg per 100 ml or greater should represent a maximum upper limit of normal for any individual For many laboratories the upper limit will be less than this

Variation in the expression of an abnormal gene for hypercholesterolemia (see below) and the possibility of multiple causes of essential hypercholesterolemia often make it necessary to use ancillary evidence to establish the diagnosis of the trait This includes the presence of xanthomatosis or the development of coronary artery disease at a relatively young age in the patient or in his family Working with population groups frequently affected with hypercholesterolemia or atherosclerosis Adlersberg et al [176] have used the presence of hypercholesterolemia in at least three family members to define the presence of the trait It is very

unusual to find a patient with xanthomatosis for whom at least a suggestive family history is not obtainable This may be a resultant of the mode of transmission (see below) Hypercholesterolemia can become manifest early in childhood, but it may not be so until the third decade or later [177]

*Plasma Lipids*

Hypercholesterolemia is nearly always associated with hyperphospholipidemia Glyceride concentrations are *usually* normal or only slightly elevated but definite hyperlipemia may be present

Plasma lipids in 20 patients with essential familial hypercholesterolemia representing 15 pedigrees are shown in Table 16-6 These patients all definitely familial represent a selected group in that they have sought medical attention Hence, the proportion having xanthomas and coronary artery disease is higher than would be obtained in a study of their kindred The distribution of their blood lipid abnormalities is representative of that seen in clinical practice

**Hyperlipemia** Six of these patients (Ri 1 Ri 2 Mi 1, Ja 2 GA, and GR) have hyperlipemic serums Nevertheless it would be erroneous to classify them as having essential familial hyperlipemia as the latter syndrome is defined in the preceding section Patients with glyceride concentrations over 3 000 to 4 000 mg per 100 ml associated with tuberous xanthomas have been reported [84 85] Some of these patients have hepatosplenomegaly abdominal pain foam cells in bone marrow and spleen and cardiovascular disease Hyperlipemia may be present in some hypercholesterolemic members of a kindred but not in others (Table 16-6) [83 84 86] Such variable patterns of hyperlipidemia are probably different expressions of the same trait but complete lipid and lipoprotein analyses on complete families are scarce and the issue is not settled Borrie [90] has seen hyperlipemia develop in a patient who previously had only hypercholesterolemia

**Phospholipids** Monoaminophosphatides (lecithin and cephalin) and diaminophosphatides (sphingomyelin) have been determined separately in several patients with essential hypercholesterolemia [21] and Thannhauser states that only the monoaminophosphatides are elevated [21] However his data show an absolute increase in the diaminophosphatides in a case of this syndrome with associated tuberous xanthoma In view of the reported preponderance of sphingomyelin in low-density lipoproteins [54 55] and the report of increased sphingomyelin levels during fasting (which may be accompanied by rise in the lower-density lipoproteins) [178] more information is needed on the phospholipid composition accompanying hyperlipemia

Schoenheimer [11] measured the dihydrocholesterol content of the sterols in a patient with essential hypercholesterolemia and found an

TABLE 16-6 CLINICAL FEATURES IN 20 CASES OF ESSENTIAL FAMILIAL HYPERCHOLESTEROLEMIA

| Patient | Age | Sex | Tendon xanthomas | Tuberous xanthomas | Coronary artery disease | TC mg/100 ml | PL mg/100 ml | TG mg/100 ml | FC/TC | TC/PL |
|---|---|---|---|---|---|---|---|---|---|---|
| St-1 | 7 | F | 0 | 0 | 0 | 468 | 368 | 146 | 0.27 | 1.27 |
| St-2 | 2[illegible] | F | 0 | 0 | 0 | 410 | 348 | [illegible]0 | 0.28 | 1.18 |
| Bi-3 | 53 | F | 0 | 0 | 0 | 43[illegible] | 447 | 0[illegible] | 0.[illegible]6 | 0.97 |
| Ja 2 | 1[illegible] | M | 0 | 0 | 0 | 355 | 284 | 3[illegible]1 | 0.[illegible]6 | 1.25 |
| TH | 41 | M | 0 | 0 | 0 | 338 | [illegible]98 | 83 | 0.[illegible] | 1.13 |
| Ni 2 | 40 | M | 0 | 0 | + | 478 | 519 | 1,307 | 0.34 | 0.82 |
| AL | 20 | F | + | 0 | 0 | 408 | 341 | 144 | 0.25 | 1.20 |
| Ni 1 | [illegible] | M | + | 0 | + | 363 | 39[illegible] | 246 | 0.24 | 0.91 |
| BA | 4[illegible] | M | + | 0 | + | 417 | 340 | 144 | 0.25 | 1.23 |
| BL | 30 | M | + | 0 | + | 336 | 289 | 125 | 0.28 | 1.19 |
| BO | 40 | M | + | 0 | + | 441 | 351 | 80 | 0.28 | 1.26 |
| CO | 43 | M | + | 0 | + | 376 | 358 | 16[illegible] | 0.27 | 1.05 |
| HO | 46 | F | + | 0 | + | 401 | 364 | 184 | 0.25 | 1.10 |
| LA | 58 | F | + | 0 | + | 454 | 3[illegible]9 | 144 | 0.27 | 1.[illegible]0 |
| PI | 44 | M | + | 0 | + | 529 | 52[illegible] | 1[illegible] | 0.28 | 1.00 |
| Ri 1 | [illegible] | M | + | + | + | 559 | 529 | 1,137 | 0.31 | 1.06 |
| Ri 2 | 50 | F | + | + | + | 68[illegible] | 571 | 1,[illegible]1 | 0.31 | 1.19 |
| GA | [illegible]0 | F | + | + | + | 664 | 554 | [illegible]44 | 0.30 | 1.20 |
| CR | 46 | M | + | + | + | 307 | 328 | 390 | 0.30 | 0.93 |
| Ja 1 | 19 | M | + | + | + | 495 | 430 | 446 | 0.29 | 1.15 |
| Mean | 40 | | | | | 443 | 40[illegible] | 388 | 0.[illegible]8 | 1.12 |
| Normals | | | | | | 240 | 2[illegible]0 | 175 | 0.2[illegible] | 0.96 |

NOTE Patients had definite familial histories otherwise they were selected at random from cases seen by the author Patients from same kindred are denoted by number TC = total cholesterol PL = phospholipids TG = triglycerides FC/TC = free/total cholesterol

Upper limits of normal from this laboratory not age adjusted

absolute increase in the small amount of this sterol which is normally present

Lipoproteins The hyperlipoproteinemia that necessarily accompanies hyperlipidemia is illustrated in Fig 16-2 Here data from studies by Gofman et al [48] have been schematically presented to emphasize the interesting tendency of different lipoprotein patterns to be accompanied



Fig 16-7 Tendon and tuberous xanthomas (patient Ja 1 Table 16-6)

by different xanthomatous manifestations Two groups of patients with essential hypercholesterolemia are presented in Fig 16-2 for clarity and emphasis Actually of course the lipoprotein changes seen in essential hyperlipidemia represent a continuum While the rather selective lipoprotein changes observed suggest that the trait does not express itself by mere amplification of the normal blood lipoprotein pattern it must be kept in mind that hypercholesterolemia induced by a number of environmental manipulations or other diseases may involve the same changes in lipoprotein classes depicted in Fig 16-2 Only the hyperlipoproteinemia associated with biliary cirrhosis may be considered to represent a truly 'diagnostic' pattern [48]

Accompanying the elevated low density lipoproteins there is significant decrease in high density lipoproteins in many patients [48 179] It has recently been suggested that this is less likely to be true in younger women with hypercholesterolemia [179]

Variation in all lipid levels in the same patient from week to week is common This is especially true of patients with accompanying hyperlipemia However some patients on isocaloric diets in a hospital environment will maintain an elevated cholesterol concentration varying by not



Fig 16-8 Tuberous xanthomas on the elbows (patient GA Table 16-6) These lesions may have an inflammatory base and resemble eruptive xanthomas (Fig 16-3)

more than 10 per cent for many weeks The hypercholesterolemia tends to increase with the age of the patient and the cholesterol concentration is affected by diet or drugs in a manner similar to that in normocholesterolemic patients

*Xanthomatosis*

The reported frequency of xanthomatosis in essential familial hypercholesterolemia varies greatly Correlation of its development with duration of hypercholesterolemia [172] but not with any critical level of hypercholesterolemia has been demonstrated There is also a poorly understood relationship between the type of xanthoma and the abnormal lipid or lipoprotein pattern

Comparison of Fig 16-2 and Table 16-6 indicates that the garden variety patient with essential familial hypercholesterolemia has elevated concentrations of primarily $S_f$ 0 to 10 and 10 to 20 lipoproteins From the chemical structure of these lipoproteins (Table 16-2) it is obvious that a

high cholesterol to phospholipid ratio and relatively normal glycerides will be found on chemical analysis If such patients have xanthomas they will usually be located on the eyelids (xanthelasma) or in tendons. Tendon xanthomas (Figs 16 6, 16 7) include both fascial and periosteal involvement

Xanthelasma has been reported to be accompanied by hypercholesterolemia in only about 50 per cent of patients Abnormal lipoprotein concentrations have been observed in a higher percentage [*1, 180*] the



Fig 16-9 Tuberous and plane xanthomas on the hands

changes being qualitatively similar to those seen in patients with only tendon xanthomas

When hyperglyceridemia is present elevated concentrations of lipoproteins of higher $S_f$ value are present (Fig 16-2) and tuberous xanthomas (Figs 16-8 16-9) tend to develop Tuberous xanthomas rarely occur with normal plasma glyceride concentrations but they may occur with tendon lesions (Fig 16 7) Whether or not hyperlipemia sufficient to cause eruptive xanthomas occurs in essential hypercholesterolemia is subject to the debate concerning classification of hyperlipemia (see above) The simultaneous occurrence of eruptive tuberous and tendon xanthomas has been observed in a rare instance [*83*]

**Extracutaneous Manifestations** Atheromatous involvement of the arteries especially the coronary arteries and of the endocardium is a

common development in essential familial hypercholesterolemia making this a potentially lethal disease This has been apparent from the earliest case reports many of which involved sudden death in children because of myocardial infarction or heart failure associated with deformed and incompetent valves The incidence of clinically detectable coronary artery disease in affected families has been reported as high as over 40 per cent [86] Clinical signs of atherosclerosis may be present without xanthomatosis Isolated cases of aortic stenosis have been attributed to hypercholesterolemic xanthomatosis [181 182] There is greater propensity to develop clinically evident coronary artery disease than cerebrovascular or peripheral vascular involvement [183]

Corneal arcus is common in this syndrome an incidence of over 50 per cent has been reported in one series of hypercholesterolemic patients [183] With the exception of corneal arcus significant extravascular xanthomatosis is not common in essential hypercholesterolemia [21] Cutaneotendinous xanthomas associated with symmetrical involvement of the central nervous system have been reported [184 185] but at least one case had no hyperlipidemia and the relationship to essential hypercholesterolemia is not clear

**Associated Findings** It has been reported that glucose and insulin tolerance test results tend to be abnormal in essential hypercholesterolemia [182] although this is not a universal experience [186] Diabetes and other endocrine disturbances do not appear more frequently in affected kindred but the possibility of a subtle relationship of carbohydrate metabolism to this syndrome has not been adequately studied

An interesting feature is the increased incidence of hyperuricemia in patients with hypercholesterolemia [187 188] the highest levels of uric acid being associated with severe xanthomatosis [188] No explanation for this association is evident Although hepatomegaly has been observed more frequently in hypercholesterolemic than normocholesterolemic members of several kindred [186] and both hepatic and splenic enlargement may accompany the hyperlipemic forms of this syndrome liver function test results are normal Gallstones and cholecystitis may be more frequent than in normocholesterolemic populations [186]

## THE BIOCHEMICAL DEFECT IN ESSENTIAL FAMILIAL HYPERCHOLESTEROLEMIA

The cause (or causes) of essential hypercholesterolemia is unknown at present Basically something is awry in the mechanisms for homeostasis of plasma lipid and lipoprotein concentrations an area poorly understood in the normal state A fascinating aspect of the syndrome is the tenacity with which abnormally high plasma concentrations of cholesterol and phospholipids are maintained If cholesterol concentrations are reduced as they sometimes can be by experimental diets containing large amounts

of unsaturated oils or experimental drugs such as estrogens sitosterols nicotinic acid or benzmalecene it is remarkable how rapidly they return to their former abnormal levels whenever the diet or drug is removed.

In many patients the abnormal cholesterol and phospholipid concentrations are quite constantly maintained This is important from the standpoint of experimental measurements of cholesterol metabolism in this syndrome since in the steady state nothing abnormal may be detected This has been the experience so far in the few measurements of plasma cholesterol turnover that have been made [189] In these patients whose plasma lipids are in equilibrium at higher concentrations it seems unlikely that cholesterol synthesis or catabolism is uniquely abnormal since one is apparently balancing the other It is possible that the biochemical basis of this syndrome lies in an increased attraction or requirement for lipid by the plasma

Since more is known about cholesterol metabolism than about phospholipid glyceride or lipoprotein metabolism it is necessary for the present to limit discussion of the possible derangement of metabolism largely to the biochemistry of cholesterol There are other possible causes of essential hypercholesterolemia of course

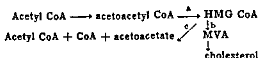
*Cholesterol Homeostasis*

For a detailed discussion of plasma cholesterol homeostasis with many references the reader is referred to that of Gould and Cook [16] The liver has been shown to be the organ primarily responsible for the maintenance of plasma cholesterol levels [190–192] and the degree of esterification [193] Since the free cholesterol of plasma and erythrocytes is in rapid equilibrium [58] it is convenient to consider the blood and liver cholesterol as a double compartment between which an important partition or gradient of cholesterol is maintained The gut is also intimately involved in cholesterol homeostasis It has been demonstrated by Hellman and coworkers that cholesterol synthesized endogenously is metabolically indistinguishable [189] from that obtained in the diet

**Cholesterol Synthesis** The pathway of synthesis of cholesterol is reviewed elsewhere in this volume (Chap 20) Synthesis of this complex molecule from a simple two-carbon precursor such as acetate involves a number of steps in which the rate of synthesis may be controlled but the exact mechanism is not yet known

The rate of hepatic cholesterol synthesis from acetate has been shown to be sensitive to a number of factors including the state of nutrition and the amount of cholesterol in the liver [19a] which in turn may be a function of that in the diet These two factors have been especially helpful in recent studies designed to localize a site of regulation [194–197] Comparison of the effects on rates of incorporation of labeled acetate and mevalonate into cholesterol indicates that the key step is probably

the conversion of β hydroxy β methyl glutaryl CoA (HMG CoA) to mevalonic acid (MVA) Bucher et al [196] have shown that fasting




decreases while factors associated with increased synthesis tend to increase the activity of the phosphopyridine nucleotide-dependent reductase (b) located in the liver microsomes The activity of the condensing (a) and cleavage (c) enzymes is not affected It therefore appears that a feed back mechanism for cholesterol homeostasis may have this enzyme locus Siperstein and Guest [197] suggest on the basis of in vitro studies that cholesterol (or its esters) does not directly mediate this effect

Many workers have established a relationship between carbohydrate utilization and cholesterol biosynthesis It has been proposed [198] that the predominant pathway of carbohydrate metabolism might be regulatory According to this hypothesis increased utilization of the hexose-monophosphate shunt productive of increased amounts of reduced TPN would lead to increased cholesterol synthesis Since diabetes is often associated with hypercholesterolemia a possible relationship of carbohydrate metabolism to essential hypercholesterolemia has been considered Wilkinson [180] found no abnormality in glucose tolerance test results in a small number of patients with the familial syndrome Waddell et al [182] who did not prepare their patients with high-carbohydrate diets preceding tests as did Wilkinson reported abnormal glucose and insulin tolerance test results in a number of patients with essential hypercholesterolemia or hyperlipemia

Synthesis of cholesterol from acetate has been measured in human beings under several conditions In hypothyroid individuals there appears to be a decided decrease in the rate of incorporation of acetate In several patients with essential hypercholesterolemia to whom labeled acetate was given by Hellman et al [189] the rate of acetate incorporation as determined by the appearance of radioactivity in the plasma did not differ from that in several other subjects without hypercholesterolemia There was also no detectable difference in the curve of decay of labeled cholesterol from the plasma

These workers also confirmed earlier observations [199] that when labeled cholesterol was fed to patients with some degree of hyperlipemia the ester cholesterol specific activity in plasma was always higher than that of the free cholesterol Normally there is a short period during which free cholesterol specific activity is higher This has been interpreted as a possible quantitative metabolic defect in essential hypercholestero-

lemia [199] It is now known however, that the free cholesterol in chylomicrons exchanges or transfers to other plasma lipoproteins much more rapidly than the esterified cholesterol [57] Incoming esterified cholesterol may therefore remain longer in plasma when chylomicrons are more slowly removed altering the usual equilibrium Whatever the mechanism of this phenomenon it has also been observed in the nephrotic syndrome, and it is unlikely to hold the key to the cause of essential hypercholesterolemia

**Cholesterol Catabolism.** Since Bloch Berg and Rittenberg first showed *that cholesterol was a precursor of the bile acids [200] a number of experiments* [103 201] have established that this pathway is the major route for cholesterol catabolism by the liver This conversion involves several steps, not all of them have yet been carried out by cell free systems and the exact biochemical mechanisms are not yet known Their complexity suggests a number of further sites where deficient activity of a single enzyme could alter cholesterol metabolism The terminal three carbons on the side chain must be removed leaving a carboxyl group at carbon 24 and the nucleus must undergo further hydroxylation This must be accompanied by reduction of the 5 6 double bond and epimerization of the 3 $\beta$ hydroxyl group to the $\alpha$ configuration common to naturally occurring bile acids Bergstrom et al have demonstrated that the first of these alterations to take place is probably located in the nucleu [103]

The principal end products of these conversions as they are excreted in the bile in man are the taurine and glycine conjugates of cholic and chenodeoxycholic acids [202 203] In the gut the conjugates are hydrolyzed and the acids may be further modified by bacterial enzymes One such modification appears to account for the deoxycholic acid frequently found in human gallbladder bile [203] and possibly the traces of lithocholic acid in bile and feces [204]

Like hepatic cholesterol synthesis catabolism to bile acids presumably is subject to sensitive control much of which may depend upon the integrity of the excretory and reabsorptive pathways involving the liver and the gut

**Excretion of Cholesterol and Its End Products** Although bile acids are *principal end products* of cholesterol catabolism in the liver the excretion of *both* acids and unchanged cholesterol into the gut represents major routes of removing cholesterol from the blood liver compartment It has been estimated that in man 1 to 2 gm cholesterol is excreted into the gut daily from the bile and that an undetermined amount is secreted in other intestinal juices or shed mucosal cells [205] Large fractions of both the sterol and bile acids excreted in bile do not depart in the feces but recycle from the gut to the liver the bile acids via the portal circulation and the sterols via the thoracic duct The amount of recycling of

bile acids is probably considerably greater than the 0 6 to 1 0 gm excreted per day in feces [205] Presumably the enterohepatic recycling of cholesterol is also considerable

The relative importance of sterol and bile acid excretion as pathways for cholesterol catabolism is not completely known Calculations of the turnover of cholesterol in the liver plasma pool in man approximate 1 0 gm per day This is based on reasonable assumptions of the amount present (12 to 15 gm) and an estimated turnover time for plasma cholesterol of about 12 days [206] It is difficult to derive a meaningful turnover rate for plasma cholesterol since it is in exchange with so many compartments at varying rates but it is interesting that such an estimate of plasma liver cholesterol turnover compares favorably with the turnover of 0 7 gm bile acids per day estimated for man by Lindstedt [207] The relative amounts of sterol and bile acids excreted in man have not been firmly established They appear to vary widely and more sterol than bile acids may be excreted in certain individuals [208 209]

It is quite apparent that interference with biliary excretion should affect cholesterol homeostasis and it has been demonstrated to do so Biliary obstruction causes hypercholesterolemia and increased hepatic synthesis of cholesterol [210 211] Simple diversion of biliary flow from the gut may have the same effect on synthetic rate and is obviated by replacement of bile into the duodenum [205]

The reabsorption of sterols from the gut is related to a number of processes with no certain assessment of the quantitative importance of each Some of these factors are degree of esterification in the gut the amount and kind of fatty acids present availability of bile and the formation of chylomicrons Cholesterol is also converted to other sterols in the gut apparently through action of bacterial enzymes the most important of the e coprostcrol is very poorly absorbed The modification of bile acids by intestinal bacteria theoretically may also affect their enterohepatic cycling

Preliminary work has suggested that the differences in plasma cholesterol obtained by feeding experimental diets differing in the degree of saturation and chain length of the fatty acids component [150] may be due to effects on reabsorption and excretion of cholesterol and its end products [212 213] From the above remarks it is obvious that there are many ways in which the reabsorption of both sterols and bile acids might be modified and cholesterol homeostasis affected Plant sterols or sitosterols which have been thought to compete with cholesterol for esterification in the gut thus inhibiting absorption represent one practical application of this theory that has enjoyed ome modest success in lowering plasma cholesterol levels On the negative side it should be noted that wide variation in cholesterol in human diets has little effect on the plasma concentration Dietary hi tories of patients with familial

hypercholesterolemia also reveal no significant difference from normocholesterolemic individuals of the same general population [186]

**The Plasma-Liver-Cholesterol Partition** There are enough examples of widely differing plasma and liver concentrations of cholesterol under both normal and abnormal conditions to indicate that the syndrome of essential hypercholesterolemia might arise primarily from a tendency to maintain an increased plasma liver lipid concentration gradient

Species differences in this gradient normally exist The rat rabbit and man for example contain about the same concentration of liver cholesterol (averaging 3 mg per gm wet weight of tissue) while man has a three to fivefold greater concentration in plasma In the rat biliary obstruction or introduction into the blood of surface-active agents causes tremendous increases in plasma cholesterol and phospholipid concentrations with either no appreciable change [210, 211] or a fall [214] in the liver concentration A similar phenomenon is present in hypercholesterolemic nephrotic rats [215]

Hepatic cholesterol synthesis is greatly increased after biliary obstruction or administration of a detergent [211 216] but the primary stimulus may be in an altered equilibrium between liver and plasma Byers Friedman and coworkers have suggested that cholate accumulating in the blood in biliary obstruction caused retention of cholesterol in the plasma They were able to induce hypercholesterolemia in rats with cholate infusion [217] Using a rather nonspecific method they have reported an increased cholate concentration in essential hypercholesterolemia and other hypercholesterolemic syndromes [218]

Frantz [219] has considered the above problem from an interesting approach assuming that the chemical activity' of cholesterol is potentially more important than its absolute concentration Thus any factor such as wetting agents tending to increase stability of lipid protein bonds or conditions altering the number of sites available for lipid transport such as an increase in lipoprotein protein or the elaboration of an abnormal protein with peculiar avidity for lipid could disturb the normal plasma liver equilibrium

**Abnormal Lipoproteins** From available data it cannot be determined whether or not the chemical composition of the various lipoprotein fractions is abnormal in essential hypercholesterolemia Ultracentrifugal fractions prepared from normal subjects and from patients with essential hypercholesterolemia suggest no distinctive alteration in the ratio of the various lipids present There is of course some interdependence of chemical composition and density of lipoproteins when the ultracentrifuge is used for isolation of fractions Lipoproteins of very abnormal lipid composition can be detected however in patients with biliary cirrhosis using existing techniques [220] Exact comparisons of the chemical nature of protein moieties in lipoproteins from normal and hyperlipidemic

subjects have not been made The localization of lipoprotein increases in all forms of hyperlipidemia to the group corresponding to low density or $\beta$ and $\alpha_1$-lipoproteins does not offer by itself a specific clue to the cause of essential hypercholesterolemia

**Phospholipid Metabolism** It has previously been mentioned that hyperphospholipidemia cannot be excluded as a possible cause of hyper cholesterolemia Rarely if ever is one seen without the other The assumption that phospholipid concentrations rise merely as a part of lipoprotein formation to permit solubilization of excessive cholesterol appears logical enough in hypercholesterolemia induced by feeding sterol to animals but it cannot be extended *a priori* to essential hypercholesterolemia Hypercholesterolemia has been induced in rats by infusion of either soybean phosphatides or neutral fat emulsions [*2-1*]

Although it is known that the liver is primarily responsible for synthesis and removal of plasma phospholipids and that various phospholipases present in mammalian tissues promote phospholipid catabolism little else is known about the homeostatic regulation of plasma phospholipid concentrations The available evidence [*38*] suggests that neither phospholipid nor cholesterol has an important function in net transport of fatty acids through the plasma and it is unlikely that high plasma concentration of either lipid is a response to a need for increased fatty acid mobilization

GENETICS

*Transmission*

The mode of transmission of the abnormal trait in essential familial hypercholesterolemia has not been satisfactorily resolved despite numerous studies There is little question that hypercholesterolemia may be transmitted as an autosomal dominant trait Lack of agreement centers upon the completeness of dominance or whether a double dose of the abnormal gene (homozygosity) leads to higher cholesterol levels developing earlier in life with accompanying severe xanthomatosis and atherosclerosis

Transmission on the basis of a simple dominant gene was first postulated by Müller [*222*] on the basis of a few cases and later by Kornerup [*223*] and Stecher and Hersh [*224*] The latter authors compared the incidence of the trait (hypercholesterolemia) as observed by Boas Parets and Adlersberg [*225*] in a large group of familial cases with the expected incidence for hereditary characters with complete penetrance They obtained a 1 1 fit consistent with one parent being heterozygous for a dominant defective allele with nearly complete penetrance and the other parent being homozygous for the recessive normal allele

In 1918 Wilkinson Hand and Fliegelman [*173*] published first hand observations on many members of several kindred and concluded that

transmission was by an incompletely dominant gene, hypercholesterolemia representing the heterozygous or "carrier" state and hypercholesterolemia with xanthomatosis representing the homozygous genotype Adlersberg Parets and Boas [68, 225] found their studies of many, smaller kindred compatible with this interpretation This was subsequently disputed by Alvord [226] Leonard [227] Piper and Orrild [172] and Harris-Jones [228], who found patients with xanthomatosis who had either offspring or a parent with "normal" cholesterol levels Similar findings exist in the data of Wheeler [229] The data of Piper and Orrild [172] were based on a follow up study of the patients of Kornerup [223] and demonstrate clearly that xanthomatosis is also a function of duration of hypercholesterolemia

Hirschhorn and Wilkinson [230] have recently published findings on three kindred which support their earlier "incompletely dominant" hypothesis As a criterion of homozygosity they now use a cholesterol level greater than 400 mg per 100 ml with or without xanthomatosis but they still imply that homozygosity is necessary for development of xanthomas They explain the failure of other workers [172 225-227] to confirm their hypothesis as erroneous definition of "normal" cholesterol levels This criticism appears justifiable for the data of Piper and Orrild [172] and Leonard [227]

It has been pointed out by Epstein Bloch Hand and Francis [231] that the crucial mating of two hypercholesterolemic nonxanthomatous persons has not been described a sufficient number of times to determine whether xanthomatosis occurs in 25 per cent of their children Thus the question of completeness of dominance cannot be answered unequivocally from all the available data Considering the secondary nature of xanthomatosis and the number of factors likely to affect penetrance of the abnormal gene the presence or absence of xanthomas may be an unfortunate index to phenotype

*Definition of Trait*

It is upon the definition of the presence or absence of the trait (hypercholesterolemia) that all studies of pedigrees depend It is now known that age [232 233] diet [160] and probably a multiplicity of other host or environmental factors [234] affect serum cholesterol levels To these factors are added spontaneous variation in individuals and variation in technical reproducibility of the cholesterol determination

In the only study of families in which an attempt has been made to define the presence of an abnormal trait by constructing age-adjusted *frequency distribution curves of serum cholesterol* concentrations Epstein et al [231] were able to demonstrate apparent bimodal frequency in male but not in female members They critically review the other genetic studies mentioned above and conclude that available

evidence does not unequivocally support any of the available genetic model

A better definition of the normal cholesterol concentration and a cleaner separation of inherited and environmental factors affecting blood lipids is needed It may be necessary to wait for means of biochemical differentiation of the trait before it can be ascertained whether ' hyper cholesterolemia is transmitted by a single gene completely or incom pletely dominant or even by multiple alleles

## TREATMENT

At pre ent there i no uniformly effective therapy for maintaining normal blood lipid concentrations in patients with essential familial hypercholesterolemia A number of medic itions have been tried which will reduce cholesterol or low density lipoprotein concentrations Often their effectiveness has been transitory undesirable side effects have developed or the preparations have been bulky and unpalatable The only form of therapy that has met the major criterion of usefulness in this syndrome i e that it be feasible throughout the life of the patient is dietary modification

### *Diet*

The dietary recommendations currently favored are based on both statistical correlations between obesity [23a] or dietary fat intake [236] and serum cholesterol levels or coronary artery di ease in large population groups and on short term experiments demonstrating the effects of dif ferent diets on serum cholesterol and lipoprotein concentrations

Basically they involve (1) maintenance of an ideal weight (2) reduc tion in the intake of fats that are largely satur ited or high in short chain fatty acids such as lard meat milk egg and coconut oil fat (3) sub stitution of more unsaturated fats such as corn cotton eed peanut afflower or fish oils The vegetable oils can be u ed for cooking and baking

The response of patients with the familial syndrome to such dietary manipulation under rigidly controlled conditions is shown in Fig 16-10 and under more practical outpatient conditions in Table 16 7

It has been advocated [151] that lipoprotein analy es form the basis for determining whether patients should be placed on high levels of unsatu rated fat or imple fat restriction depending on the predominant lipo protein fraction which is elevated This more sophisticated plan is based on the observation of increa es in glyceride concentrations (and very low-den ity lipoproteins) on a very high-carbohydr ite intake

Skim milk products hould be included in these diets as sources of calcium and protein and vitamin upplementation is indicated for initial reducing diets There is no evidence to upport addition of pecial prepara

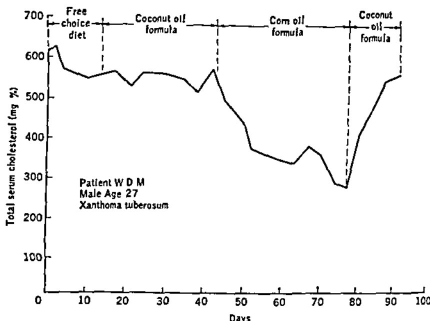



Fig 16-10 Effect of different fats comprising 60 per cent of total calories fed as isocaloric liquid formulas on serum cholesterol in a 27 year-old male with essential hypercholesterolemia Triglycerides were normal during period of study (*Data courtesy of Dr Daniel Steinberg*)

TABLE 16-7 COMPARISON OF SERUM CHOLESTEROL LEVELS IN MILLIGRAMS PER 100 ML OBTAINED WITH DIFFERENT DIETS IN OUT-PATIENTS WITH ESSENTIAL HYPERCHOLESTEROLEMIA

| *Patient* | *Diet* A† | *Diet* B† | *Diet* C† |
|---|---|---|---|
| M L | 509 ± 32 | 420 ± 23 | 454 ± 24 |
| F T | 338 ± 3 | 255 ± 18 | 251 ± 8 |
| W B | | 353 ± 14 | 368 ± 5 |
| I C | | 449 ± 26 | 420 ± 12 |
| C G | | 332 ± 16 | 317 ± 16 |

* Each diet was isocaloric prepared at home according to prearranged menus and was maintained until the cholesterol level was stable for 3 to 4 weeks Each cholesterol value represents the mean of 3 to 10 determinations

† Diets A 50 to 70 gm daily of saturated fat (milk eggs meat) B 20 gm of such saturated fat C 20 gm saturated fat plus 30 to 50 gm corn or safflower oil Carbohydrate was adjusted to accommodate changes in fat content Diet C permitted a normal carbohydrate intake (*Study conducted in cooperation with Dr Duncan McCollester*)

tions of vitamins or purified es ential fatty acids The mechanism whereby such dietary changes affect serum cholesterol level is not known but there is no evidence that they are potentially harmful

*Drugs*

Drugs that have been used include both hormones and compounds designed to inhibit cholesterol synthesis speed its catabolism or prevent reabsorption from the gut All estrogenic preparations will cause an increase in high-density lipoproteins and usually some decrease in total cholesterol when administered to hypercholesterolemic patients of either sex Feminizing doses must be used and gynecomastia and lo s of libido occur in males with distressing frequency Gastrointestinal distress water retention and uterine bleeding also restrict the use of estrogens in females These effects cannot be selectively avoided by simultaneous administration of androgens or progesterone [237] and no estrogen analogue is yet available which offers dissociation of the lipid and other hormone effects

Thyroxine or many of its analogues will lower levels of both cholesterol and low density lipoproteins [237 238] when administered to euthyroid hypercholesterolemic subjects This frequently cannot be maintained without u ing doses sufficient to raise basal oxygen consumption a circumstance not without potential hazard to patients with coronary artery disease [237]

There is no universal agreement about the effects of ACTH and adrenocorticoids on serum lipids although large doses (100 to 200 mg per day) of cortisone have been found to lower the cholesterol in hypercholesterolemic patients [237] Other metabolic actions of these hormones make them impractical for long term therapy

Nicotinic acid in daily doses sufficiently large (3 to 5 gm) to induce vasodilatation and gastrointestinal distress has been reported to lower cholesterol quite significantly in some but not all hypercholesterolemic patients [239] Buffered salts may alleviate some of the ide effects [240]

Sitosterol preparations such as Cytellin (Lilly) in doses of 25 gm per day will produce a small decrease in serum cholesterol in many hypercholesterolemic patients [241] The required high dosage of this drug and it u ual failure to restore chole terol concentration to normal have limited its acceptance

A potent inhibitor of cholesterol biosynthe i MER 29 has recently become available for treatment of hypercholesterolemia It apparently blocks the last step in cholesterol synthe is the reduction of desmosterol (24 dehydrocholesterol) [242] Since MER 29 admini tration is accompanied by accumulation of desmo terol in pla ma [242] the therapeutic value of the drug remains to be establi hed

A number of drugs have had a vogue in treatment of hypercholes-

terolemia but have been dropped for lack of dramatic success These include potassium iodide, choline inositol and methionine

In patients with elevated glycerides and tuberous xanthomas, sodium heparin given subcutaneously in doses of 100 mg per day, or 200 mg three times a week may cause a reduction in hyperlipemia and in the size of the cutaneous xanthomas Chronic heparin therapy is expensive and not easily adapted to outpatient use Its use in hyperlipidemia without cardiovascular complications is not recommended at present

## SUMMARY

1 Essential familial hypercholesterolemia is an inheritable tendency toward development of hyperlipidemia and hyperlipoproteinemia. Depending upon the duration and severity of this manifestation cutaneotendinous xanthomas and atherosclerosis may secondarily develop Death may occur at an early age from the complications of atheromatous involvement of the endocardium or the coronary arteries or there may be no limitation of the normal life expectancy

2 In addition to hypercholesterolemia and hyperphospholipidemia plasma glycerides may be elevated The hyperlipidemia is associated with increased concentrations of low density lipoproteins

3 The disease is presumed to represent an abnormality in the homeostatic mechanisms for regulating plasma cholesterol concentrations The specific defect is unknown

4 The hypercholesterolemic trait is apparently inherited as an autosomal dominant Expression of this trait is influenced by a number of host and environmental factors including age and diet This makes it difficult to determine precisely the incidence and penetrance of the trait Two genetic models have been proposed (1) that the gene is completely dominant (2) that the gene is incompletely dominant with the homozygous genotype more likely to develop severe hyperlipidemia and xanthomatosis than the heterozygote

## BIBLIOGRAPHY


1 Gofman J W Rubin L McGinley J I and Jones H B Hyperlipoproteinemia Am J Med 17 514 1954
2 Standard nomenclature of diseases and operations Suppl II J A M A 166 377 1958
3 *Manual of the International Statistical Classification of Diseases Injuries and Causes of Death* vols I and 2 World Health Organization Geneva 1957
4 Sperry W M and Webb M A revision of the Schoenheimer Sperry method for cholesterol determination J Biol Chem 187 97 1950
5 Bragdon J H Colorimetric determination of blood lipids J Biol Chem 190 153 1951
6 Rapport M M and Alonzo N J Photometric determination of fatty acid ester groups in phospholipids J Biol Chem 217 193 1955

7 Stewart C P and Hendry E B Th pho pl olipins of blood Biochem J 29 1683 1935
8 Hirsch J and Ahrens E H Jr The separation of complex lipid mixtures by the use of silicic acid chromatography J Biol Chem 233 311 1958
9 Dole V P A relationship between non-esterified fatty acids in plasma and the metabolism of glucose J Clin Invest 35 150 1956
10 Gordon R S Jr and Cherkes A Unesterified fatty acid in human blood plasma J Clin Invest 35 206 1956
11 Schoenheimer R Über eine Störung der Cholesterin Ausscheidung Ztschr klin Med 132 749 1933
12 Kelsey F E and Longenecker H E Distribution and characterization of beef plasma fatty acids J Biol Chem 139 727 1941
13 Lipsky S R Haavik A Hooper C L and McDivitt R W The biosynthesis of the fatty acids of the plasma of man I The formation of certain chromatographically separated higher fatty acid of the major lipid complexes from acetate-1 C¹ J Clin Invest 36 233 1957
14 Sperry W M The relationship between total and free cholesterol in human blood serum J Biol Chem 114 125 1936
15 Kritchevsky D *Cholesterol* Wiley New York 1958
16 Cook R P (Ed) *Cholesterol* Academic Press Inc New York 1958
17 Friedman M Byers S O and St George S Cholesterol metabolism Ann Rev Biochem 25 613 1956
18 Popják G Biosynthesis of cholesterol and related substances Ann Rev Biochem 27 533 1958
19 Taurog A Entenman C and Chaikoff I L The choline-containing and noncholine-containing phospholipids of plasma J Biol Chem 156 385 1944
20 Phillips G B The isolation and quantitation of the principle phospholipid components of human serum using chromatography on silicic acid Biochim et biophys acta 29 594 1958
21 Thannhauser S J *Lipidoses Diseases of the Cellular Lipid Metabolism* 2 ed Oxford New York 1950 3d ed Grune & Stratton New York 1958
21a Svennerholm E and Svennerholm L Determination of glucolipids in plasma Acta chem scandinav 10 1048 1956
22 Hanahan D J The phospholipid in *Chemistry of Lipids as Related to Atherosclerosis* edited by I H Page Charles C Thomas Springfield Ill 1958
23 Phillips G B Lipid composition of human serum lipoprotein fraction with density greater than 1 21 Proc Soc Exper Biol & Med 100 19 1959
24 Feulgen R and Bersin T Zur Kenntnis des Plasmalogens IV Mitteilung Eine neuartige Gruppe von Phosphatiden (Acetalphosphatide) Ztschr phys ol Chem 260 217 1939
25 Rapport M M Lerner B Alonzo N Franzl R E Structure of plasmalogen II J Biol Chem 225 859 1957
26 Debuch H Nature of the linkage of the aldehyd residue in natural plasmalogens Biochem J 67 261 1957
27 Gray G M The structure of the plasmalogens of ox heart Biochem J 70 425 1958
28 Marinetti G V and Erbland J The structure of pig heart plasmalogen Biochim et biophys acta 26 429 1957
29 Carlson L A and Wadstrom L B On the occurrence of tri di and mono glycerides in human serum Blood lipids and the clearing factor *Kon Kl Vla n Acad Wetenschap Lette e Scho e Kunsten B lg Jaa boek* p 123 1956
30 Carlson L A and Wadstrom L B Studies on the glycerides during the clearing reaction Clin chim acta 2 9 1957
31 Goodman D S The interaction of human serum albumin with long-chain fatty acid anions J Am Chem Soc 80 389 1958
32 Goodman D S and Shafrir E The interaction of human low density lipoproteins with long-chain fatty acid anions J Am Chem Soc 81 364 1959
33 Goodman D S Interaction of human erythrocytes with sodium palmitate J Clin Invest 37 1 ) 1958

34 Fredrickson D S McCollester D L and Ono K The role of unesterified fatty acid transport in chylomicron metabolism J Clin Invest 37 1333 1958
35 Dole V I Fractionation of plasma non-esterified fatty acids Proc Soc Exper Biol & Med 93 532 1956
36 Dole V P James A T Webb J I W Rizack M A and Sturman M F The fatty acid patterns of plasma lipids during alimentary lipemia J Clin Invest 38 1544 1959
37 Hirsch J Farquhar J W Peterson M L and Stoffel W The fatty acid composition of adipose tissue in man J Clin Invest 38 1011 1959
38 Fredrickson D S and Gordon R S Jr Transport of fatty acids Physiol Rev 38 585 1958
39 Gordon R S Jr Unesterified fatty acid in human blood plasma II The transport function of unesterified fatty acid J Clin Invest 36 810 1957
40 Laurell S Turnover rate of unesterified fatty acids in human plasma Acta physiol Scandinav 41 158 1957
41 Fredrickson D S and Gordon R S Jr The metabolism of albumin bound C[14] labeled unesterified fatty acids in normal human subjects J Clin Invest 37 1504 1958
42 Gordon R S Jr and Cherkes A Production of unesterified fatty acids from isolated rat adipose tissue incubated *in vitro* Proc Soc Exper Biol & Med 97 150 1958
43 White J E and Engel F L Lipolytic action of ACTH on adipose tissue *in vitro* J Clin Invest 37, 1556 1958
44 Raben M S and Hollenberg C H Effect of growth hormone on plasma fatty acids J Clin Invest 37 922 1958
44a Blankenhorn D H Carotenoids in man J Biol Chem 227 963 1957
44b Cohen H Observations on carotenemia Ann Int Med 48 219 1958
45 Macheboeuf M Recherches sur les phosphoaminolipides et les stérides du sérum et du plasma sanguins Bull soc chim biol 11 268 485 1929
46 Lindgren F T Elliott H A and Gofman J W The ultracentrifugal characterization and isolation of human blood lipids and lipoproteins with applications to the study of atherosclerosis J Phys & Colloid Chem 55 80 1951
47 deLalla O and Gofman J Ultracentrifugal analysis of human serum lipoproteins in *Methods of Biochemical Analysis* edited by D Glick vol I Interscience New York 1954
48 Gofman J W deLalla O Glazier F Freeman N K Lindgren F T Nichols A V Strisower E H and Tamplin A R The serum lipoprotein transport system in health metabolic disorders atherosclerosis and coronary artery disease Plasma 2 413 1954
49 Havel R J Eder H A and Bragdon J H The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum J Clin Invest 34 1345 1955
50 Hillyard L A Entenman C Feinberg H and Chaikoff I L Lipide and protein composition of four fractions accounting for total serum lipoproteins J Biol Chem 214 79 1955
51 Kunkel H G and Slater R J Lipoprotein patterns of serum obtained by zone electrophoresis J Clin Invest 31 677 1952
52 Gage S H and Fish P A Fat digestion absorption and assimilation in man and animals as determined by the dark field microscope and a fat soluble dye Am J Anat 34 1 1924
53 Bragdon J H Havel R J and Boyle E Human serum lipoproteins I Chemical composition of four fractions J Lab Clin Med 48 36 1956
54 Steele J M and Kayden H J The nature of the phospholipids in human serum and atheromatous vessels Tr A A Physicians 68 249 1955
55 Phillips G B The phospholipid composition of human serum lipoprotein fractions separated by ultracentrifugation J Clin Invest 38 489 1959
56 Kunkel H G and Bearn A G Phospholipid studies of different serum lipoproteins employing P[32] Proc Soc Exper Biol & Med 86 887 1954
57 Fredrickson D S McCollester D L Havel R J and Ono K The early

steps in transport and metabolism of exogenous triglyceride and cholesterol in *Chemistry of Lipides as Related to Atherosclerosis* edited by I H Page Charles C Thomas Springfeld Ill 1958
58 Hagerman J S and Gould R G The *in vitro* interchange of cholesterol between plasma and red cells Proc Soc Exper Biol & Med 78 329 1951
59 Gitlin D Cornwell D G Nakasato D Oncley J L Hughes W L and Janeway C Studies on the metabolism of plasma proteins in the nephrotic syndrome J Clin Invest 37 172 1958
60 Avigan J H Eder H A and Steinberg D Metabolism of the protein moiety of rabbit serum lipoproteins Proc Soc Exper Biol & Med 95 4 9 1957
61 Rodbell M and Fredrickson D S The nature of the proteins associated with dog and human chylomicrons J Biol Chem 234 562 1959
62 Lindgren F T Freeman N K Nichols A V and Gofman J W Blood lipids and the clearing factor *Kon nkl Vlaam Acad Wetenschap Lette en Schone Kunsten België Jaarboek* p 224 1956
63 Marsh J B Net synthesis of low density plasma l poproteins by rat liver slices Fed Proc 17 270 1958
64 Radding C M Feigelson E and Steinberg D Synthesis of serum lipoproteins by rat liver slices Fed Proc 18 1?3 1959
65 Haft D E White A Rohein I S Walcott G Maresca L and Eder H A Relationship between synthesis of cholesterol and plasma lipoproteins in th isolated perfused rat liver Fed Pro 18 240 1959
66 Rodbell M Fredrickson D S and Ono K Metabolism of chylomicron proteins in the dog J Biol Chem 234 567 1959
67 Crocker A C Skin xanthomas in childhood Pediatrics 8 573 1951
68 Adlersberg D Hypercholesteremia with predisposition to atherosclerosis Am J Med 11 600 1951
69 Klatskin G Familial xanthomatoses and arcus senilis New Internat Clinics 3 13 1941
70 Bürger M and Grütz O Über hepatosplenomegale Lipoidose mit xanthomatosen Veranderungen in Haut und Schleimhaut Arch Dermat u Syph 166 542 1932
71 Holt L E Aylward F X and Timbres H G Idiopathic familial lipemia Bull Johns Hopkins Hosp 64, 279 1939
72 Levy B M Idiopathic lipemia J Pediatrics 26 367 1946
73 Poulson H M Familial lipemia new form of lipoidosis showing increase in neutral fats combined with attacks of acute pancreatitis Acta med scandinav 138 413 1950
74 Bruton O C and Kanter H J Idiopathic familial hyperlipemia A M A Am J Dis Child 82 153 1951
75 Harslof F Idiopathic familial lipemia attended with hepatosplenomegaly Acta med scandinav 130 140 1948
76 Hetzel P S Idiopathic hyperlipemia with a report of two cases occurring in one family and a review of the literature M J Australia 2 396 1951
77 Klatskin G and Gordon M Relationship between relapsing pancreatitis and essential hyperlipemia Am J Med 11 3 1951
78 Gadrat J Bazex A Dupre Douste Blazy A Xanthomes miliarres et très forte hyperlipidémie évoluant en poussées declenchées par une alimentation hyperlipidique Bull soc franç dermat et syph 359 1952 clinical data abstracted from Lever W F Smith P A J and Hurley N A ref 83
79 Gaskins A L Scott R B and Kessler A D Report of three cases of idiopathic familial hyperlipemia use of ACTH and cortisone Pediatrics 2 480 1953
80 Soffer A and Murray M Prolonged observations of the cardiovascular status in essential hyperlipemia Circulation 10 255 1954
81 Fredrickson D S Unpublished data
82 Kennedy R L J and Collett R W Chronic relapsing pancreatitis and hyperlipemia Am J D Child 78 80 1949
83 Lever W F Smith P A J and Hurley N A Idiopathic hyperlipemia and

primary hypercholesteremic xanthomatosis I Clinical data and analysis of the plasma lipids J Invest Dermat 22 33 1954
84 Malmros H Swahn B and Truedsson E Essential hyperlipemia Acta med scandinav 149 91 1954
85 Schettler G Eggstein M and Jobst H Essential hyperlipemia Ger Med Monthly 3 310 1958
86 Adlersberg D Inborn errors of lipid metabolism clinical genetic and chemical aspects A M A Arch Path 60 481 1955
87 Bohman M Hyperlipemi ar okand genes (2 fall) Nord med 46 10 6 1951
88 Christensen S Dollerup E and Eskjaer-Jensen S Idiopathic hyperlipemia latent diabetes mellitus and severe neuropathy Acta med scandinav 161 5 1958
89 Adlersberg D and Wang C Syndrome of idiopathic hyperlipemia mild diabetes mellitus and severe vascular damage Diabetes 4 210 1955
90 Borrie Peter Essential hyperlipemia and idiopathic hypercholesterolemic xanthomatosis Brit M J No 5050 911 Oct 19 1957
91 Hirschhorn K Hirschhorn R Fraccaro M and Book J A Incidence of familial hyperlipemia Science 129 716 1959
92 Joyner C R Essential hyperlipemia Ann Int Med 38 759 1953
93 Cadrat J L hyperlipémie essentielle Bull soc franç dermat et syph 474 1954
94 Opitz H Hochgradige Lipamie unklarer Genese bei einem 12 jahrigen Knaben Deutsche med Wchnschr 61 88 1935
95 Lever W F Smith P A J and Hurley N A Idiopathic hyperlipemia and primary hypercholesteremic xanthomatosis II Analysis of the plasma proteins and lipids by means of electrophoresis and fractionation of the plasma proteins effects of high speed centrifugation and of extraction with ether on the plasma proteins and lipids J Invest Dermat 22 53 1953
96 Chapman F D and Kinney T D Idiopathic lipemia Am J Dis Child 62 1014 1941
97 Rohn R J Gandek C and Bartley M D Severe hyperlipemia associated with non-diabetic pregnancy report of a case Arch Int Med 82 339 1948
98 Schultze W H Über grosszellige Hyperplasie der Milz bei Lipoidaemie Verhandl deut ch path Gesellsch 15 47 1912
99 Warren S and Root H F Lipoid containing cells in the spleen in diabetes with lipemia Am J Path 2 69 1926
100 Bernstein S S Williams H H Hummel F C Shepherd M D and Erickson B N Metabolic observation on a child with essential hyperlipemia J Pediat 14 570 1939
101 Dunphy E B Ocular conditions associated with idiopathic hyperlipemia Tr Am Ophth Soc 47 197 1949
102 Bloomfield A L and Shenson B The syndrome of idiopathic hyperlipemia with crises of violent abdominal pain Stanford M Bull 5 185 1947
103 Bergstrom S and Borgstrom B Metabolism of lipids Ann Rev Biochem 25 177 1956
104 Frazer A C Fat absorption and its disorders Brit M Bull 14 212 1958
105 Bloom B Chaikoff I L Reinhardt W O Entenman C and Dauben W C The quantitative significance of the lymphatic pathway in transport of absorbed fatty acids J Biol Chem 184 1 1950
106 French J E and Morris B The removal of C labelled chylomicron fat from the circulation in rats J Physiol 138 326 1957
107 Morris B and French J The uptake and metabolism of C labeled chylomicron fat by the isolated perfused liver of the rat Quart J Exper Physiol 43 180 1958
108 Bragdon J H and Gordon R S Jr Tissue distribution of C after the intravenous injection of labeled chylomicrons and unesterified fatty acids in the rat J Clin Invest 37 574 1958
109 Havel R J and Fredrickson D S The metabolism of chylomicra I The

removal of palmitate 1 C14-labeled chylomicra from dog plasma J Clin Invest 35 1025 1956

110 Tattrie N H Positional distribution of saturated fatty acids on egg lecithin J Lipid Research 1 60 1959

111 Bergstrom S Borgstrom B and Rottenberg M Intestinal absorption and distribution of fatty acids and glycerides in the rat metabolism of lipids 3 Acta physiol scandinav 25 120 1952

112 Morris B The exchange of lipids between the circulating plasma and the hepatic lymph Blood lipids and the clearing factor Koninkl Vlaam Acad Wetenschap en Schone Kunsten België Jaarboek p 311 1956

113 Fawcett D W Observations on the cytology and electron microscopy of hepatic cells J Nat Cancer Inst 15 1475 1955

114 Jones H B Gofman J W Lindgren F T Lyon T P Graham D M Strisower B and Nichols A V Lipoproteins in atherosclerosis Am J Med 11 358 1951

115 McCandless F and Zilversmit D B Fate of triglycerides and phospholipids of lymph and artificial fat emulsions disappearance from the circulation Am J Physiol 193 294 1958

116 Korn E D Clearing factor a heparin activated lipoprotein lipase J Biol Chem 215 1 15 1955

117 Engelberg H Human endogenous lipemia clearing activity Studies of lipolysis and effects of inhibitors J Biol Chem 222 601 1956

118 Jeffries G H The effect of fat absorption on the interaction of chyle and plasma in the rat Quart J Exper Physiol 39 77 1954

119 Korn E D Inactivation of lipoprotein lipase by heparinase J Biol Chem 226 827 1957

120 Korn E D and Quigley T W Studies on lipoprotein lipase of rat heart and adipose tissue Biochim et biophys acta 18 143 1955

121 Jeffries G H The sites at which plasma clearing activity is produced and destroyed in the rat Quart J Exper Physiol 39 261 1954

122 Waddell W R and Geyer R P Effect of insulin on clearance of emulsified fat from the blood in depancreatized dogs Proc Soc Exper Biol & Med 96 251 1957

123 Albrink M J Fitzgerald J R and Man L B Reduction of alimentary lipemia by glucose Metabolism 7 162 1958

124 Havel R J Evidence for the participation of lipoprotein lipase in the transport of chylomicrons Blood lipids and the clearing factor Koninkl Vlaam Acad Wetenschap en Schone Kunsten België Jaarboek p 265 1956

125 Thannhauser S J and Stanley M M Serum fat curves following oral administration of I 131 labeled neutral fat to normal subjects and those with idiopathic hyperlipemia Tr A Am Physicians 62 245 1949

126 Crofford O B Congalio J G Meng H C and Newman E V Demonstration of the specific metabolic defect in primary essential hyperlipemia with secondary diabetes J Clin Invest 37 886 1958

127 Lever W F and Waddell W R Idiopathic hyperlipemia and primary hypercholesteremic xanthomatosis VII Effect of intravenously administered fat on the serum lipids J Invest Dermat 25 233 1955

128 Lever W F and Klein I *The inhibition of lipemia clearing by hyperlipemic serum* J Invest Dermat 29 465 195[illegible]

129 Fredrickson D S and Gordon R S Jr Unpublished observations

130 Carlson L A and Olhagen B *Studies on a case of essential hyperlipemia* Blood lipids with special reference to the composition and metabolism of the serum glycerides before during and after the course of a viral hepatitis J Clin Invest 38 8[illegible] 1959

131 Albrink M J Man E B and Peters J P The relation of neutral fat to lactescence of serum J Clin Invest 34 14[illegible] 1955

132 Waddell W R Geyer R P Hurley N and Stare F J Abnormal carbohydrate metabolism in patients with hypercholesterolemia and hyperlipemia Metabolism 7 [illegible] 1958

133 Kennedy E P Metabolism of lipids Ann Rev Biochem 26 119 1957
134 Borgstrom B in Chemistry of Lipides as Related to Atherosclerosis edited by I H Page p 185 Charles C Thomas Springfield Ill 1958
135 Lehninger H L Oxidation of fatty acids in Chemistry of Lipids as Related to Atherosclerosis edited by I H Page p 265 Charles C Thomas Springfield Ill 1958
136 Langdon R The biosynthesis of fatty acids in cell free preparations of mammalian liver in Chemistry of Lipids as Related to Atherosclerosis edited by I H Page p 291 Charles C Thomas Springfield Ill 1958
137 Shapiro B Lipid dynamics in adipose tissue in Progress in the Chemistry of Fats vol 4 Pergamon Press London 1957
138 Stetten DeW Jr and Salcedo J Jr The source of the extra liver fat in various types of fatty liver J Biol Chem 156 27 1945
139 Rudman D and Seidman F Lipemia in the rabbit following injection of pituitary extract Proc Soc Exper Biol & Med 99 146 1958
140 Seifter J and Baeder D H Role of liver on consequences of lipid mobilization Proc Soc Exper Biol & Med 95 747 1957
141 Chalmers T M Kekwick A and Pawan G L S On the fat mobilizing activity of human urine Lancet No 7002 866 Apr 26 1958
142 Scow R O Chernick S S and Guarco B A Ketogenic action of pituitary and adrenal hormones in pancreatectomized rats Diabetes 8 132 1959
143 Friedman M Rosenman R H and Carrol V Changes in serum cholesterol and blood clotting time in men subjected to cyclic variation of emotional stress Circulation 17 852 1958
144 Franklin S N Splenomegaly with lipemia Proc Roy Soc Med 30 711 193
145 Goodman M Shuman H and Goodman S Idiopathic lipemia with secondary xanthomatosis hepatosplenomegaly and lipemia retinalis J Pediat 16 596 1940
146 Kuo P T Whereat A F and Horwitz O The effect of lipemia upon coronary and peripheral arterial circulation in patients with essential hyperlipemia Am J Med 26 68 1959
147 Regan T J Binak K Gordon S DeFazio V and Hellems H K The modification of myocardial blood flow and oxygen consumption during post prandial lipemia and heparin induced lipolysis J Clin Invest 38 1033 1959
148 Greig H B W Inhibition of fibrinolysis by alimentary lipaemia Lancet 271 16 1956
149 O Brien J R Fat ingestion blood coagulation and atherosclerosis Am J M Sc 234 373 1957
150 Ahrens E H Jr Nutritional factors and serum lipid levels Am J Med 23 928 1957
151 Gofman J W Diet in prevention and treatment of myocardial infarction Am J Cardiol 1 271 1958
152 Wilkinson C F Jr Space fat feeding a regime of management for familial hyperlipemia Ann Int Med 45 674 1956
153 Lever W F Herbst F S M and Hurley N A Idiopathic hyperlipemia and primary hypercholesteremic xanthomatosis IV Effect of prolonged administration of heparin on the serum lipids in idiopathic hyperlipemia A M A Arch Dermat 71 150 1955
154 Rayer P F O Traité théorique et pratiques des maladies de la peau Paris 1836
155 Addison T and Gull W On a certain affection of the skin vitiligoidea a) plana b) tuberosa with remarks Guy s Hosp Rep 7 265 1851
156 Wilkes (1868) cited by Bloom D Kaufman S R and Stevens R A Hereditary xanthomatosis Arch Dermat & Syph 44 1 1942
157 MacKenzie S Tr Path Soc London 33 370 1882 cited by Levin E A and Sullivan M Familial xanthoma Arch Dermat & Syph 33 967 1936
158 Lehzen G and Knauss K Über Xanthoma multiplex planum tuberosum molluscIforme Arch path Anat 116 85 1889
159 Ehrmann Beitr klin Chir 4 341 1889 cited by Wile U J and Duemling W W Familial xanthoma Arch Dermat & Syph 21 642 1930

160 Fogge C H General xanthelasma or vitiligoidea Tr Path Soc London 24 24? 18?? 
161 Fox T C A case of xanthelasma multiplex Lancet 2 688 187J
16? Poen gen A Mittheilung eines seltenen Falles von Xanthela ma multiplex Arch path Anat 91 350 1883
163 Arning E Ein Fall von familiare Xanthomatose Arch Dermat u Syph 105 ?90 1J10
164 Chauffard A and LaRoche G Pathogénie du xanthélasma Semaine méd 30 241 1910
165 Pick L and Pinkus F Weitere Untersuchungen zur Xanthomfrage die echten xanthomatosen Neubeldungen Dermat Ztschr 36 18 7 190J
166 Politzer S and Wile U J Xanthoma tuberosum multiplex J Cutan Dis 30 235 191?
167 Burns F S A contribution to the study of the etiology of xanthoma multiplex Arch Dermat & Syph 2 415 19?0
168 Rowland R S Xanthomatosis and the reticuloendothelial system Arch Int Med 42 611 19?8
169 Polano K Über die Pathogenese der Cholesteren der Haut Arch Dermat u Syph 174 213 1936
1 0 Montgomery H and Osterberg A I Xanthomatosis correlation of clini al histopathologic and chemical studies of cutaneous xanthoma Arch Dermat & Syph 37 373 1938
1 1 Thannhauser S J and Magendantz H The different clinical groups of xanthomatous diseases a clinical physiological study of 22 cases Ann Int Med 11 1662 1938
1?2 Piper J and Orrild L Essential familial hypercholesterolemia and xanthomatosis Am J Med 21 34 1956
1 3 Wilkinson C F Hand L A and Fliegelman M T Essential familial hypercholesterolemia Ann Int Med 29 671 1948
174 Gofman J W Glazier F Tamplin A Strisower B and deLalla O Lipoproteins coronary heart disease and atherosclerosis Physiol Rev 34 589 1954
175 McGinley J Jones H and Gofman J Lipoproteins and xanthomatous diseases J Invest Dermat 19 71 1952
176 Adlersberg D Schaefer L E and Drachman S R Incidence of hereditary hypercholesteremia J Lab & Clin Med 39 237 1952
177 Adlersberg D Schaefer L E Steinberg A G and Wang C Genetic aspects of idiopathic hypercholesteremia Studies of patients and offspring in 200 randomly selected families Circulation 10 600 1954
1?8 Peterson V P Plasma phospholipids during prolonged starvation in man Acta med scandinav 143 249 195?
1 9 Hood B and Angervall G Studies in essential hypercholesterolemia and xanthomatosis Am J Med 26 30 1959
180 Epstein N N Rosenman R H and Gofman J W Serum lipoproteins and cholesterol metabolism in xanthelasma A M A Arch Dermat & Syph 65 ?0 195
181 Barr D P Rothbard S and Eder H A Atherosclerosis and aortic stenosis in hypercholesteremic xanthomatosis J A M A 156 943 1954
182 Boas F I Lister S K and Adlersberg D Calcific aortic stenosis study of rum cholesterol Am Heart J 48 485 1954
183 Gurarich J L Familial hypercholesteremic xanthomatosis a preliminary report Am J Med 26 8 1959
184 von Bogaert L Froelich A and Epstein F Une deuxième observation de cholestérinose tendineuse symétrique avec symptoms cérébraux Ann méd 42 9 193?
185 Froehlich A L Les Xanthomatoses Les Éditions Acta medica belgica Bruxels, 1951
186 Wilkinson C F Essential familial hypercholesterolemia cutaneous metabolic and hereditary aspects Bull N Y Acad Med 26 6?0 1950
18 Adlersberg D Newer advances in gout Bull N Y Acad Med 25 651 1949

188 Harris Jones J N Hyperuricemia and essential hypercholesterolemia Lancet 1, 857 1957
189 Hellman L Rosenfeld R S Eidinoff M L Fukushima D K Gallagher T F Wang C I and Adlersberg D Isotopic studies of plasma cholesterol of endogenous and exogenous origins J Clin Invest 34 48 1955
190 Friedman M Byers S O and Michaelis F Production and excretion of cholesterol in mammals IV Role of liver in restoration of plasma cholesterol after experimentally induced hypocholesteremia Am J Physiol 164 789 1951
191 Gould R G Campbell D J Taylor C B Kelly F B Jr Warren I and Davis C B Jr Origin of plasma cholesterol using C[14] Fed Proc 10 191 1951
192 Hotta S and Chaikoff I L The role of the liver in the turnover of plasma cholesterol Arch Biochem & Biophys 56 28 1955
193 Friedman M and Byers S O Observations concerning the production and excretion of cholesterol in mammals XVI The relationship of the liver to the content and control of plasma cholesterol ester J Clin Invest 34 1369 1955
194 Tomkins G M and Chaikoff I L Cholesterol synthesis by liver I Influence of fasting and of diet J Biol Chem 196 569 1952
195 Frantz I D Schneider H S Hinkelman B T Suppression of hepatic cholesterol synthesis in the rat by cholesterol feeding J Biol Chem 206 465 1954
196 Bucher N L R Overath P and Lynen F Enzymes controlling cholesterol biosynthesis in livers of fasting rats Fed Proc 18 20 1959
197 Siperstein M D and Guest M J Studies on the homeostatic control of cholesterol synthesis J Clin Invest 38 1043 1959
198 Siperstein M D and Fagan V M Studies on the relationship between glucose oxidation and intermediary metabolism I The influence of glycolysis on the synthesis of cholesterol and fatty acids in normal liver J Clin Invest 37 1185 1958
199 Biggs M W and Coleman D A quantitative metabolic defect in lipid metabolism associated with abnormal serum lipoproteins in man Circulation 7 393 1953
200 Bloch K Berg B N and Rittenberg D The biological conversion of cholesterol to cholic acid J Biol Chem 149 511 1943
201 Siperstein M D Jayko M E Chaikoff I L and Dauben W J Nature of the metabolic products of C[14] cholesterol excreted in bile and feces Proc Soc Exper Biol & Med 81 720 1952
202 Siperstein M D and Murray A W Cholesterol metabolism in man J Clin Invest 34 1449 1955
203 Norrantz J C and Sjovall J Bile acids in newborn and adult humans Acta chem scandinav 11 1032 1957
204 Mosettig E Heftmann E Sato Y and Weiss E Occurrence of lithocholic acid in feces in healthy men Science 128 1433 1958
205 Bergstrom S The formation and metabolism of bile acids under different conditions in *Hormones and Atherosclerosis* edited by G Pincus p 31 Academic Press Inc New York 1959
206 London I M and Rittenberg D Deuterium studies in normal man I The rate of synthesis of serum cholesterol II Measurement of total body water and water absorption J Biol Chem 184 687 1950
207 Lindstedt S Turnover of cholic acid in man *Acta physiol scandinav* 40 1 1957
208 Frantz I D Carey J B Moss R Eckert J F Goldfarb M and Katz H I Observations on the turnover of cholesterol in a human subject Minnesota Med 41 157 1958
209 Avigan J and Steinberg D Metabolism and excretion of cholesterol in man Fed Proc 18 5 1959
210 Byers S O and Friedman M Observations concerning the production and excretion of cholesterol in mammals V The relation of biliary retention of cholesterol distention of the biliary tract the shunting of bile to the vena cava and the removal of the gastrointestinal tract to the hypercholesteremia consequent on biliary obstruction J Exper Med 95 19 1952

211 Iredrickson D S Loud A V Hinkelman B T Schneider H S and Frantz I D The effect of ligation of the common bile duct on cholesterol synthesis in the rat J Exper Med 99 43 19o4
212 Hellman L Rosenfeld R S Insull J and Ahrens L H Jr Intestinal excretion of cholesterol a mechanism for regulation of plasma level J Clin Invest 36 888 19o7
°13 Haust H L and Beveridge J M R Effect of varying type and quantity of dietary fat on the fecal excretion of bile acids in humans subsisting on formula diets Arch Biochem & Biophys 78 36" 1958
14 Hirsch R L and Kellner A The pathogenesis of hyperlipemia induced by means of surface active agents I and II J Exper Med 104 1 1o 19o6
15 Ro enman R H Friedman M and Byers S O The distribution of cholesterol and total lipids in the nephrotic rat J Clin Invest 34 ,00 19o5
216 Frantz I D Jr and Hinkelman B T Acceleration of hepatic cholesterol synthesis by Triton WR 1339 J Exper Med 101 2?5 1955
17 Byers S O Friedman M Biggs M W and Gunning B Ob ervations concerning the production and excretion of cholesterol in mammals IX The mechanism of the hypercholesterolemic effect of cholic acid J Exper Med 97 511 1953
°18 Friedman M Byers S O and Rosenman R H The accumulation of serum cholate and its relation hip to hypercholesteremia Science 115 313 1952
1) Frantz I D Jr Cholesterol metabolism Minnesota Med 38 7 ) 1955
°°0 Eder H A The lipoproteins of human serum Am J Med 23 26) 19 "
°1 Friedman M and Byers S O Role of hyperphospholipidemia and neutral fat increase in plasma in the pathogenesis of hypercholesteremia Am J Physiol 186 13 1956
°°° Muller Carl Xanthomata hypercholesterolemia angina pectoris Acta med scandinav Suppl 89 ,5 1938
°3 Kornerup V Familiaer Hypercholesteroaemia og Xanthomatose Kolding Denmark 1948
°°4 Stecher R M and Hersh A H Note on genetics of hypercholesterolemia Science 109 61 1949
°5 Boas I P Parets A D and Adlersberg D Hereditary disturbance of cholesterol metabolism A factor in the genesis of atherosclerosis Am Heart J 35 611 1948
°°6 Alvord R M Coronary heart disease and xanthoma tuberosum associated with hereditary hyperlipemia study of 30 affected persons in family Arch Int Med 84 100_ 1)49
°° Leonard J C Hereditary hypercholesterolaemic xanthomatosis Lancet 2 1 3) 19o6
8 Harr s-J nes J N Jones E G and Wells P G Xanthomatosis and essential hypercholesterolaemia Lancet 1 8o5 195,
) Wheeler I O The genetic a pect of athero clero i Am J Med 23 653 195,
30 Hirschhorn K and Wilkinson C F The mode of inheritance in essential familial hypercholesterolemia Am J Med 26 60 19o9
31 Epstein F H Block W D Hand L A and Francis T F Jr Familial hypercholesterolemia xanthomatosis and coronary heart disease Am J Med 26 3) 1959
3 Keys A Mickelsen O Miller E v O Hayes E R and Todd R L The concentration of cholesterol in the blood of normal man and its relation to age J Clin Invest 29 134 1950
33 Linholm H Stu les n norm al a lults for variation in s rum lipids with sex age relati e body weight and with body buil l Scandinav J Clin & Lab I est Suppl 8 3 19o6
34 Adlersberg D and Schaefer I F Lditorial Th nt rplay of he edity and environment in the regulation of circulating lipi l and in atherogenesis Am J Med 26 1 19 9
°35 Dawber T R Moore F E and Mann G V Coronary heart disease in the Framingham study Am J Publ Health Suppl p 4 Apr l 19o

236 Keys A Kuakawa A Bronte-Stewart B Larson M and Keys M D Lessons from serum cholesterol studies in Japan Hawaii and Los Angeles Ann Int Med 48 83 1958
237 Oliver M F and Boyd G S Hormonal aspects of coronary artery disease in *Vitamins & Hormones* vol 16 p 147 Academic Press Inc New York 1958
238 Strisower B Gofman J W Galioni E F Almada A A and Simon A Effect of thyroid extract on serum lipoproteins and serum cholesterol Metabolism 3 218 1954
239 Altschul R Haffer A and Stephen J D Influence of nicotinic acid on serum cholesterol in man Arch Biochem & Biophys 54 558 1955
240 Altschul R and Hoffer A Effects of salts of nicotinic acid on serum cholesterol Brit M J 2 713 1958
241 Best M M Duncan C H van Loon E J and Wathen J D The effects of sitosterol on serum lipids Am J Med 19 61 1955
242 Avigan J Steinberg D Thompson M J and Mosettig E Mechanism of action of MER 29 an inhibitor of cholesterol biosynthesis Biochem Biophysical Res Comm 2 63 1960

# Chapter 17

# Infantile Amaurotic Family Idiocy

---

*Donald S Fredrickson*

## GENERAL ASPECTS OF THE SPHINGOLIPIDOSES

Three diseases characterized by the intracellular accumulation of a different sphingolipid are now recognized Each is possibly due to an inheritable defect in the metabolism of its typical lipid but this is unproved These diseases are known clinically as infantile amaurotic family idiocy (Tay Sachs disease) Niemann Pick disease (Chap 18) and Gaucher s disease (Chap 19) they may be differentiated chemically as ganglioside sphingomyelin and cerebroside lipidoses respectively

Further research may add to this list other familial conditions in which sphingolipids accumulate in tissues These include Pfaundler Hurler disease (gargoylism) and certain of the degenerative brain diseases known as the leukodystrophies Despite the accumulation of gangliosides within the central nervous system Pfaundler Hurler disease is still considered to be primarily a disease of mucopolysaccharide metabolism [1 2] The leukodystrophies may also be accompanied by lipid accumulation and similar histochemical lesions but distinctive chemical features have not been uncovered

The three familial sphingolipidoses involve either nervous or reticuloendothelial (RE) tissues The abnormalities in Tay Sachs disease are confined to the nervous system Niemann Pick and Gaucher s disease may involve only the viscera or they may be accompanied by extensive brain damage One of many features of these diseases which remain to be determined is the influence of local tissue factors on expression of the abnormal biochemical trait

Although chemical analyses have proved that the sphingolipidoses are distinct entities techniques are still inadequate to distinguish possible subvarieties of each disease The sporadic detection of less than subtle chemical differences associated with the same clinical and morphologic

findings portends further subclassification and provides a warning against categorical interpretation of some of the older case histories or the most recent theories of pathogenesis

It is 35 years since the youngest of the sphingolipidoses (Niemann Pick disease) became a recognized clinical entity [3] and fully 60 years have passed since Thudichum disposed of "protagon" as the supposed single chemical constituent of brain and isolated sphingosine and cerebrosides from that tissue [4] The pace of the search for understanding of the sphingolipidoses has not been rapid, the route passing over the difficult terrain of neurochemistry and through a dark forest of exceedingly complex compounds has been most forbidding There is now a *period of greater expectation Continuing development of techniques for* microchemical analysis of nervous tissue brain biopsy chromatography of lipids and study of enzymes involved in lipid metabolism provide assurance that these diseases may soon be understood at the molecular level

At present not all the sequential steps in sphingolipid synthesis and breakdown are known and precise information concerning possible enzymatic defects causing these diseases is not available

## INFANTILE AMAUROTIC FAMILY IDIOCY

### DEFINITION

Amaurotic family idiocy (AFI) is a disease of the central nervous system characterized *clinically by blindness paralysis and dementia* and histologically by abnormal ganglion cells filled with gangliosides and possibly other lipids demyelination and proliferation and lipid loading of glial cells

The symptoms of AFI may begin very early or later in life When the onset is in infancy the disease is known commonly as Tay-Sachs disease This form is associated with the characteristic cherry red spot in the retina and is invariably fatal usually within 1 to 3 years To describe the later appearing forms a variety of chronologic or eponymic designations has been employed A late infantile form (Jansky Bielschowsky) has been recognized Lying between this form and the adult (Kufs) variety is a juvenile (Vogt Spielmeyer) form which has been most extensively studied in Scandinavia Usually becoming manifest at 5 to 10 years of age *the juvenile form differs from the infantile in* certain clinical features and especially in racial distribution and genetic pattern [5 6] Although the ganglioside accumulation in the brain is definitely characteristic for the infantile variety [7–16] the issue is not yet settled for the other forms of AFI [7 8 15] Unless otherwise indicated further discussion will be restricted to the infantile variety or Tay Sachs disease

## HISTORICAL ASPECTS

In 1881 Tay a British ophthalmologist described a cherry red spot in the fundus of an infant and 3 years later a similar lesion in two siblings [17] In 1887 Sachs an American neurologist reported clinical and pathologic observations of an infant with blindness and dementia [18] Within a few years he observed a total of eight cases with the typical retinal change and described the syndrome using the name *amaurotic family idiocy* [19 20] A large number of cases has been subsequently reported especially from institutions having a high proportion of Jewish patients Aronson and coworkers have recently summarized clinical and genetic data concerning more than 100 cases [21 21a] and a number of other comprehensive reviews have appeared [22–25]

In 1939–1942 Klenk [7–11] made the important discovery that the brain in infantile AFI contained greatly increased amounts of glycolipids containing a substance later identified as N acetylneuraminic acid or sialic acid (see below) Klenk termed these lipids gangliosides to distinguish them from the cerebrosides which also contain sphingosine fatty acid and hexose but not neuraminic acid or its derivatives

Before it was known that the lipids involved in infantile AFI and Niemann Pick disease were different the similarity of the morphologic changes in the brain and retina in both diseases led some observers notably Pick and Bielschowsky [26] to consider the two conditions as different forms of the same disease This view is now known to be incorrect Although these two rare diseases have been reported as possibly occurring in the same sibship [27] it is probable that they have no etiologic relationship beyond chemical similarities in the offending lipids Some points of similarity between both these conditions and Gaucher s disease (cerebrosidosis) and Pfaundler Hurler syndrome (a mucopolysaccharide gangliosidosis) have also already been mentioned

## CLINICAL FEATURES

### *Onset*

Infantile AFI commonly becomes manifest at 4 to 6 months of age in children previously appearing healthy and normal Typical cases may become apparent much earlier In one family two siblings had symptoms at birth and died at 18 days and 7 weeks respectively [28 29] Onset of symptoms may be noted as late as 2½ years of age [30] No significant correlation has been discovered between occurrence of the disease and order of birth or other prenatal or early postnatal environmental influences The disease is much more common among Jewish infants than in those of any other parentage

*Symptoms and Signs*

AFI often becomes evident insidiously beginning with listlessness or weakness retardation in development or difficulties in feeding The signs progress slowly but relentlessly to further motor deterioration The earliest sign may not be neurodepressive but neuroirritative such as hyperacusis [21] In 73 cases studied by Kanof et al [21] the most common initial symptom was hyperacusis

Visual difficulties may be suspected quite early because of inattentiveness fixed gaze or other abnormal eye movements Although the fundus may be normal in the first months of life the changes in the macula leading to the typical cherry red spot soon become evident On rare occasions this sign has not been observed but other "equivalent" changes in the macular area have been present Optic atrophy is common

As the neurologic changes increase in severity epileptiform seizures or convulsions may occur and the child may reach a state of decerebrate rigidity The average age at onset is about 6 months and the average duration of the disease about 24 months [21a]

The clinical course of the disease has been divided into several arbitrary time phases by Aronson and his coworkers [21 21a] in order to correlate with certain interesting morphologic changes which are observed as these children survive longer than about 15 months Up to that time the cranial measurements are normal and the brain is slightly subnormal in weight After this time the brain actually may increase in size and the cranial measurements become concomitantly larger than normal

## ANATOMIC CHANGES

*Nervous System*

Important pathologic changes in AFI are limited to the nervous system The brain may be abnormally small normal in size or large In patients dying at a very early age the entire brain is usually atrophic and there is moderate ventricular dilatation [21] When the onset is later or the rate of progression slower the cerebral expansion may be considerable with associated marked atrophy of the cerebellum and the brain stem Regardless of size the brain is frequently firm or leathery in consistency

The essential histologic features are as follows (1) The ganglion cells are enormously swollen or ballooned and distorted Most of their nuclei are displaced or disintegrated and the cytoplasm fails to show the usual chromophilic Nissl substance The cytoplasm is sometimes vacuolated and apparently filled with material taking some but not all lipid stains (2) There is demyelination or arrested myelination of the fibers related to these nuclei (3) The glial cells proliferate and are converted to foam or fat granule cells These changes progress sequentially and seem

to increase in severity the longer these patients survive Far advanced disease is associated with extreme glial hyperplasia cystic degeneration of the white matter patchy encephalomalacia and meningeal fibrosis [24] In this late stage the leptomeninges may be swollen gelatinous and loaded with foam cells Thrombi which may be present in cerebral vessels possibly arise from the expulsion of lipid from overloaded endothelial cells into the vessel lumen [24]

The cherry red spot is produced by lesions in the cells in the retinal layer identical to those occurring in the ganglion cells in the brain [31] The characteristic sign consists of a white zone in the macular region in which the fovea is seen as a small red spot The grayish macula is thought to be due to edema and swelling of the internuclear layers on either side of the fovea [32] or to swelling and necrosis of the ganglion cells which are most numerous in the region of the macula [22] Demyelination and degeneration of the optic nerve also occur The cherry red spot is also seen frequently in Niemann Pick disease The histologic features of the retinal lesion are practically identical to those in infantile AI I They have been described extensively by Goldstein and Wexler [32]

The ontogenetically most recent areas of the brain are the most susceptible to the disease process [24] The spinal cord may have similar degenerative changes which are sometimes of such severity that only the axis cylinders remain devoid of their myelin sheaths

**Histochemical Features** The abnormal ganglion cells in the brain and spinal cord or outside the central nervous system such as in the sympathetic ganglia or myenteric plexus cannot be distinguished by ordinary hematoxylin eosin staining from those in Niemann Pick infantile Gaucher s or Pfaundler Hurler diseases Even with the use of all available differential stains it may be most difficult to distinguish gangliosides Diezel [33 34] has reported successful delineation of the lipid in ganglion cells in various neuronal lipidoses In the AI I brain he has found acidic lipid which is PAS positive sudanophilic and metachromatic in the ganglion and glial cells Using a modified Bial s test for neuraminic acid he concludes that gangliosides may be demonstrated within these cell in large quantities Diezel has also reported that some of the acidic glycolipid in the glial and ganglion cells is extracted with difficulty and has interpreted this as strong protein binding of the lipid [34] Others have expressed caution concerning the interpretation that neuraminic acid in tissues is necessarily present in gangliosides [35] The staining properties of complex lipids may also differ considerably when they are compared as pure substances and in fixed tissue preparations [36]

*Pathologic Changes in Other Tissues*

After Pick and Bielchowsky [96] expressed the opinion that infantile AI I and Niemann Pick disease were possibly different manifestations

of the same disease pathologists searched extensively for signs of visceral involvement in AFI. There is now universal agreement that the severe involvement of the RE system outside the brain characteristic of Niemann-Pick disease does not occur in AFI. There are isolated cases, however, in which lipid accumulation in viscera [37] or hepatic or splenic enlargement has been reported. Diezel [34] reports that in one case of AFI he found small amounts of glycolipid in RE cells of the spleen and liver. No case otherwise typical of AFI appears to have been reported, however, in which ganglioside accumulation outside the central nervous system has been demonstrated by chemical analyses.

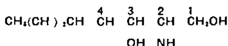
## GANGLIOSIDES

### *Chemistry*

Gangliosides contain sphingosine, fatty acid, hexose, hexosamine, and sialic acid. The common constituent of the sphingolipids is ceramide, the name given to N-acyl sphingosine.

**Sphingosine.** The long chain dihydroxy amine base sphingosine was discovered by Thudichum in his classical studies of the composition of brain, which began in 1874 and culminated in his monograph *Die chemische Konstitution des Gehirns des Menschen und der Tiere*, published in 1901.[1] The actual structure of sphingosine was unknown to Thudichum and has only recently been elucidated by a number of studies which have been summarized by Carter [39, 40].

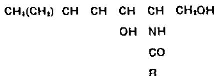
The base as isolated from tissue sphingolipids is mainly D-*erythro*-1,3-dihydroxy-2-amino-4-*trans*-octadecene [40].




The configurational aspects of this molecule may take on special significance as information concerning its biochemistry is unfolded. *Erythro* refers to the configuration of the substitutions on carbons 2 and 3, *trans* to the configuration of the 4,5 double bond, and D- to the configuration of carbon 2. A variable amount of base in naturally occurring sphingolipids is present as the saturated compound dihydrosphingosine [40, 41]. Recent gas chromatographic analyses of the periodate oxidation products of sphingosine isolated from human plasma lipids indicate that as much as 10 per cent of the base may be dihydrosphingosine and that there may be other long chain bases of unknown composition similar to sphingosine [42].

[1] The thoroughly annotated biography of Thudichum by Drabkin [38] provides a fascinating description of this scientist and the struggle for recognition of his achievements.

**Ceramide** In the sphingolipids sphingosine is always present as the N acyl derivative in which a long chain fatty acid is attached to the 2 amino group through an acid amide linkage (ceramide)




Ceramide

A ceramide lignocerylsphingosine has been isolated from extracts of liver lung and spleen [43–45] but there are no quantitative data available concerning either normal or possibly pathologic concentrations of ceramides in tissues

The fatty acid (R) differs among the sphingolipids and in the same class of sphingolipid in different tissues The predominant acids so far demonstrated include palmitic and stearic acid and the $C_{22}$ and $C_{24}$ acids such as behenic lignoceric nervonic and cerebronic acids

The gangliosides isolated from either normal or AFI brains by Klenk contained largely stearic acid and smaller amounts of lignoceric and nervonic acids On the other hand lignoceric and nervonic predominate in beef spleen gangliosides [10–12 46–48]

The composition of the ceramide fatty acids has often been established solely by melting point determinations Some of the results must be considered tentative and information concerning possible minor components of most of the fatty acids is insufficient The application of gas chromatography to this problem will increase our knowledge considerably

The more hydrophilic group attached to the ceramide through the C-1 hydroxyl group provides the distinguishing characteristic of each of the different sphingolipids In sphingomyelin this is phosphorylcholine (Chap 18) in cerebrosides a hexose (Chap 19) and in gangliosides the more complex poly saccharide chain containing sialic acid and hexosamine

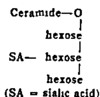
The hydrophilic lipid which Klenk found in such large quantities in the infantile AFI brain had two peculiar properties (1) its strong color reaction with Bial s orcinol reagent typical of furfural or its derivatives (2) its tendency to decompose to black humin substances on heating with mineral acid Klenk called this lipid substance X Its characteristics had been observed earlier by others [49 50] in normal brain and other tissues but in much smaller amounts

It was soon recognized [9–11] that substance X contained ceramide and hexose but was distinguished from cerebroside by the additional presence of the Bial reactive substance a polyoxyamino acid To the over all glycolipid Klenk gave the name *ganglioside* [11] and to the acid the

name *neuraminic acid* [9] It was determined by Blix et al [51, 52] and confirmed by Klenk [12] that gangliosides also contained hexosamine

As discussed below, neuraminic acid is now known to be present in gangliosides as N acyl derivatives referred to generically as sialic acid

The structure of gangliosides is still somewhat indefinite In gangliosides of brain and spleen the molar ratio of fatty acid sialic acid and hexose (including hexosamine) is usually 1 1 3 [48] Klenk has recently reported that gangliosides from these tissue sources may be mixtures of hexosamine-containing and hexosamine-free compounds [48] A general structural formula for the basic unit as proposed by Klenk [12 48] is as follows




The third hexose may be replaced by acylated hexosamine The hexose units may be either glucose or galactose Study of the hydrolysis products of certain ganglioside-containing mucolipids (see below) suggests that glucose may be the first hexose unit attached to the ceramide [53 54]

TABLE 17 1 HEXOSAMINE AND SIALIC ACID COMPOSITION OF BRAIN GANGLIOSIDES (GRAMS PER 100 GM DRY WEIGHT)

| *Material* | *Hexosamine* | *N-acetyl sialic acid* | *N-acetyl sialic acid per hexosamine* |
|---|---|---|---|
| Infantile brain age 2 years | | | |
| Cortex cerebri | 0 15 | 0 48 | 3 2 1 |
| White matter | 0 04 | 0 10 | |
| Senile brains (mean of ?0 samples) | | | |
| Cortex cerebri | 0 13 | 0 51 | 3 8 1 |
| White matter | 0 012 | 0 095 | |
| Tay-Sachs brain age 1¾ years | | | |
| Cortex cerebri | 1 09 | 2 15 | 2 0 1 |
| Nucleus caudatus | 0 66 | 1 40 | 2 1 1 |
| White matter | 0 39 | 0 14 | 2 8 1 |

Source L Svennerholm [16] reprinted with permission of Blackwell Scientific Publications Oxford

Of all the lipids in brain only gangliosides appear to contain hexosamine [16] Svennerholm [16] found higher ratios of hexose to hexosamine and sialic acid to hexosamine in gangliosides prepared from senile brains than in those isolated from an infant brain He also found a low ratio of sialic acid to hexosamine in the gangliosides from a single case of infantile AFI (Table 17 1) This led him to advance the interesting hypothesis that

loss of hexosamine may be a normal step in the degradation of gangliosides and that such a mechanism may be deficient in AFI

*Macromolecular Structure of Gangliosides*

There is some evidence that gangliosides may be present in brain as macromolecules or polymers of repeating units This hypothesis gains some support from the isolation of lipids of high molecular weight which contain large amounts of sialic acid hexose and ceramide

One of these lipids is strandin which has been extensively studied by Folch Pi and associates [53 55] A minimal molecular weight of 2 000 to 3 000 and a particle size of greater than 250 000 have been suggested for strandin [53] and it is maintained that it may have a larger amount of sialic acid than pure gangliosides [53] Some preparations but not all contain peptide material

Rosenberg and Chargaff [56–59] have introduced the term *mucolipid* largerly to cover material similar to strandin which they isolate from brain by techniques derived from those of Folch Pi They have isolated from brain a mucolipid suggestive of a highly polymerized ganglioside Analysis of this material obtained from beef brain indicated a molecular weight of about 180 000 For each mucolipid molecule there were about 100 molecules of sphingosine and fatty acid 234 of hexose (galactose to glucose 8 1) 36 of N acetylgalactosamine and 151 of sialic acid Amino acids were also present They have also found certain differences between the mucolipid isolated from normal brain compared with that from infantile AFI brain The latter has different solubility properties lacks the peptide material usually found with the normal mucolipid and is more metachromatic [59]

Bogoch [54] has also proposed a structure for gangliosides in brain as polymers of a basic repeating unit All workers in this area agree however that there is insufficient evidence for conclusions as to the natural state of these lipids and that the definition of these compounds is still largely one of technique of isolation

*Sialic Acid*

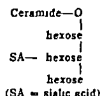
The characteristic acid in gangliosides has recently become a subject of intensive investigation reaching far beyond the area of the sphingo lipids Before Klenk had described neuraminic acid Blix had isolated a similar compound from bovine submaxillary gland mucin and had given it the name *sialic acid* [60] Intensive work in both their laboratories and in those of others including Gottschalk in Australia and Yamakawa in Japan resulted in the isolation of these compounds from a variety of tissues and secretions mostly bound to the carbohydrate portion of mucoproteins

Although Klenk had originally isolated neuraminic acid from ganglio-

name *neuraminic acid* [9] It was determined by Blix et al [51, 52] and confirmed by Klenk [12] that gangliosides also contained hexosamine

As discussed below neuraminic acid is now known to be present in gangliosides as N acyl derivatives referred to generically as sialic acid

The structure of gangliosides is still somewhat indefinite In ganglio ides of brain and spleen the molar ratio of fatty acid sialic acid and hexose (*including hexosamine*) *is usually 1 1 3* [48] *Klenk has recently reported* that gangliosides from these tissue sources may be mixtures of hexosamine-containing and hexosamine-free compounds [48] A general structural formula for the basic unit as proposed by Klenk [12 48] is as follows




The third hexose may be replaced by acylated hexosamine The hexo e units may be either glucose or galactose Study of the hydrolysis products of certain ganglioside-containing mucolipids (see below) suggests that glucose may be the first hexose unit attached to the ceramide [53 54]

TABLE 17 1 HEXOSAMINE AND SIALIC ACID COMPOSITION OF BRAIN GANGLIOSIDES (GRAMS PER 100 GM DRY WEIGHT)

| *Material* | *Hexosamine* | *N-acetyl sialic acid* | *N-acetyl sialic acid per hexosamine* |
|---|---|---|---|
| Infantile brain age 2 years | | | |
| Cortex cerebri | 0 15 | 0 48 | 3 2 1 |
| White matter | 0 01 | 0 10 | |
| Senile brains (mean of 20 samples) | | | |
| Cortex cerebri | 0 13 | 0 51 | 3 8 1 |
| White matter | 0 012 | 0 095 | |
| Tay Sachs brain age 1¾ years | | | |
| Cortex cerebri | 1 09 | 2 15 | 2 0 1 |
| Nucleus caudatus | 0 66 | 1 40 | 2 1 1 |
| White matter | 0 39 | 0 14 | 2 8 1 |

Source L Svennerholm [16] reprinted with permission of Blackwell Scientific Publications Oxford

Of all the lipids in brain only gangliosides appear to contain hexosamine [16] Svennerholm [16] found higher ratios of hexose to hexosamine and sialic acid to hexosamine in gangliosides prepared from senile brains than in those isolated from an infant brain He also found a low ratio of sialic acid to hexosamine in the gangliosides from a single ca e of infantile AFI (Table 17 1) This led him to advance the interesting hypothe is that

gangliosides [16] it is apparent that both the extraction procedure and the sialic acid method must be as specific as possible The extraction and determination of ganglioside of brain including the adaptation of column chromatography for this purpose [16] have been critically reviewed by Svennerholm

A most sensitive and specific color reaction using thiobarbituric acid has been developed recently by Warren [66] This method will no doubt replace older and less sensitive techniques employing orcinol resorcinol diphenylamine Ehrlich s reagent and other chromogenic substances

## METABOLISM OF SPHINGOSINE AND GANGLIOSIDES

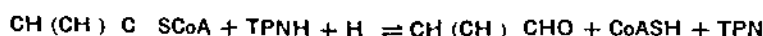
Parts of the synthetic pathways for ganglioside synthesis may be constructed from interpolations among recently demonstrated enzymatic reactions These include specifically the synthesis of sphingosine [67 68] of sialic acid [69] and of other glycolipids (cerebrosides) Undoubtedly other reactions involved at some point in the pathway such as the formation of hexosamines are the same as for formation of other carbohydrates The interesting sequence of steps by which the hydrophilic groups are attached and removed from the ceramide and the possible role of mucoproteins or mucopolysaccharides as donors and receptors of the hexose moieties remain to be elucidated

### *Synthesis of Sphingosine*

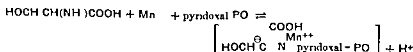
As partially suggested by in vivo experiments of Zabin and Mead [70] and Sprinson and Coulon [71] and recently demonstrated in vitro by Brady and coworkers [67 68] the synthesis of sphingosine in brain is accomplished by the condensation of a 16 carbon chain (palmitaldehyde) with a 2 carbon fragment derived from serine Acetate is a precursor by way of palmitate and formate or glycine by way of serine

Cell free preparations of brain from immature rats readily incorporate serine and either palmitaldehyde or palmityl CoA into sphingosine Palmitaldehyde is utilized in the absence of CoA with palmityl CoA TPNH is required In this system the following sequence of reactions has been proposed by Brady Formica and Koval [68]

Palmityl CoA is reduced to palmitaldehyde

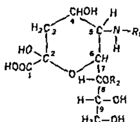



A Schiff base–metal complex is formed with serine pyridoxal and $Mn^{++}$ ions



sides as the methoxy derivative [61] it has become apparent that the compound is naturally *present as the N acetyl, N glycolyl or N O-*diacetyl derivatives In human brain gangliosides the sialic acid appears to be mainly the acetyl derivative in beef spleen and horse erythrocytes the glycolyl derivative predominates [48]

It has been proposed [62] that sialic acid be used as the group name for these derivatives and that *neuraminic acid be reserved for the basic* unsubstituted compound The structure of neuraminic acid as proposed by Gottschalk [63] and recently established [64] is shown in Fig 17 1




| | | $R_1$ | $R_2$ |
|---|---|---|---|
| Neuraminic acid | | H | H |
| Sialic acid | N Acetylneuraminic acid | $-\overset{O}{\overset{\|}{C}}-CH_3$ | H |
| | N Glycolylneuraminic acid | $-\overset{O}{\overset{\|}{C}}-CH_2OH$ | H |
| | N O Diacetylneuraminic acid | $-\overset{O}{\overset{\|}{C}}-CH_3$ | $-\overset{O}{\overset{\|}{C}}-CH_3$ |

Fig 17 1 The structure of neuraminic acid and its naturally occurring derivatives (*By permission of L Warren [65]*)

**Distribution of Sialic Acid** Sialic acid is present in the body in mucoproteins in many tissues and the extracellular fluid As a result of its wide distribution and the demonstration of at least one interesting immunochemical function for sialic acid the literature concerning these compounds is considerable It has been reviewed in part by Gottschalk [63] and by Cornforth et al [64] One widely studied reaction *is the cleavage* of a terminal neuraminic acid unit from the erythrocyte mucoproteins by a neuraminidase present in the cholera vibrio or the influenza virus *Cells so* treated are no longer subject to agglutination by the influenza virus This indicates that sialic acid must function as a prosthetic group for the agglutination

**Determination of Sialic Acid** The determination of sialic acid in suitable extracts is the present basis for determination of gangliosides Since the amount of protein bound sialic acid in brain is abo[ut] that in

that is recoverable in the aqueous phase of a chloroform methanol extract has been studied Radin Martin and Brown [77] found that $C^{14}$-galactose was rapidly incorporated into this fraction after a single injection and that the radioactivity subsequently declined with a half life of about 8 days The neutral glycolipids (cerebrosides) were also rapidly labeled and the subsequent decline in radioactivity occurred more slowly than in the ganglioside fraction Mandelstam and Burton [78] observed incorporation of both uniformly labeled D-glucose and D galactose into gangliosides The rates were age dependent and the maximum incorporation occurred 6 to 10 days postnatally This is much earlier than the time of maximum incorporation of these hexoses into cerebrosides in rat brain [77] The distribution of glucose and galactose in the gangliosides was similar Most was present as galactose or galacto amine relatively little as glucose or neuraminic acid and almost none was found in the ceramide moiety

Judging from the rapid utilization of hexose injected intraperitoneally either glucose or galactose enters the brain from the blood and is incor porated intact into the hexose or hexosamine portions of the gangliosides and cerebrosides The enzymes necessary for the utilization of uridine nucleotides including the UDP galactose 4 epimerase necessary for the interconversion of glucose and galactose are present in brain [79 80]

It is conceivable that the hexoses are not incorporated directly into single ganglioside molecules but first enter a polysaccharide template Ceramide and sialic acid could then be joined to form a ganglioside polymer Since there is no agreement that gangliosides naturally exist in macromolecular form [48] this mechanism is entirely speculative

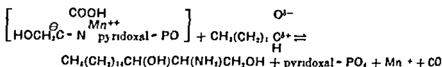
It is of interest that erythrocytes [12 81] and spleen [12] and many other tissues contain glycolipids whose proposed structures appear to lie between those classically representative of gangliosides on the one hand and cerebrosides on the other Klenk has postulated that these lipids may represent transition compounds occurring by alteration in the hydrophilic group attached to the basic ceramide [12] These possible interrelationships between gangliosides and the other sphingolipids may take on more significance when the biochemistry of the developing brain is considered

### LIPIDS AND THE DEVELOPING NERVOUS SYSTEM

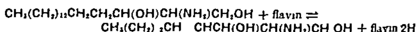
Lipids make up much of the substance of the brain and although their role may be primarily structural they are nevertheless essential to proper function of the neurons The classes of lipids in brain are summarized in Table 17 2 [82]

The brain is far from a finished product at birth and one of the most significant changes during the postnatal development is the increase

Condensation then occurs with decarboxylation of the serine-pyridoxal complex



The resulting dihydrosphingosine is oxidized presumably catalyzed by a flavoprotein



**Ceramide Synthesis** The conversion of sphingosine to ceramide may be the next step in sphingolipid synthesis and presumably involves a reaction between sphingosine and a long-chain fatty acid thiolester of coenzyme A [72] Zabin [73] has reported incorporation of serine and palmityl CoA into ceramide by a rat brain homogenate That ceramides are possible common intermediates in the synthesis of sphingolipids was first postulated a number of years ago by Thannhauser and coworkers [43 74] It has been recently demonstrated that ceramide is an intermediate in sphingomyelin synthesis [72 75] (Chap 18)

**Sialic Acid** The N acetylneuraminic acid present in gangliosides is probably synthesized from pyruvate and N acetylhexosamine although the reaction has not yet been demonstrated in brain Comb and Roseman [69] have purified an enzyme from *Clostridium perfringens* which cleaves pyruvate from human serum sialic acid, leaving N acetyl D-mannosamine The reaction is reversible and under the available conditions the enzyme does not catalyze incorporation of either acetylglucosamine or acetylgalactosamine into sialic acid This interesting finding of mannosamine in human sialic acid is not necessarily at variance with the previously mentioned synthesis of a compound identical to naturally occurring sialic acid from pyruvate and N acetyl D-glucosamine [64] The synthetic conditions used by Cornforth Firth and Gottschalk [64] *would favor some epimerization of glucosamine to mannosamine* It is postulated that the synthesis of sialic acid from pyruvate (or more readily oxalacetate) and hexosamine proceeds by an intramolecular aldol condensation [64]

**Elaboration of Ganglioside** The further steps by which ceramide hexose hexosamine and sialic acid are converted to ganglioside remain to be determined It has been demonstrated that uridine diphosphogalactose is the primary intermediate in the synthesis of brain cerebrosides [76] (Chap 19) this may very well be true for ganglioside synthesis as well

*The in vivo incorporation* of labeled hexoses into rat brain gangliosides represented by the nondialyzable and sialic acid-containing glycolipid

In the ganglion cell gangliosides appear in both the nucleus and cyto pla m [88]

The typical lipids of the white matter are cholesterol sphingomyelin and cerebrosides [84 85 89 90] and possibly phosphatidyl serine [87] In the period of active myelination of the brain (10 to 40 days of age in the rat [91] and at least 1 to 2 years in the human being [92]) the total brain content of these lipids increases markedly [84 85 90] The lipid in myelin is laminated with protein about the axons of peripheral nerves by the enveloping Schwann cell membrane [93] In the brain there are no Schwann cells and it has been presumed without proof that the oligodendroglia assume the function of myelination [94]

The change in lipid content of the human brain with age is illustrated in Tables 17 1 [16] and 17 3 [15] Although cerebroside has been reported in gray matter (Table 17 3) the gangliosides which also contain hexose must be completely removed from the ' cerebroside fraction to avoid falsely high values for the latter Folch Pi for example found in mice that the white matter contributed practically all the cerebroside in the brain [85] Robins et al [87] also found very low concentrations of cerebroside in either layer of the monkey cerebellar cortex

Cerebroside should therefore be considered as typical of the myelin sheath as ganglioside is of the ganglion cell In keeping with these findings is the difference in age at which maximal incorporation of hexose into these two lipids takes place In rat brain the maximum synthesis of gangliosides occurs between 6 and 10 days [78] and of cerebrosides between 10 and 20 days [76] Klenk [12] also considers sphingomyelin an almost exclusive constituent of the myelin sheath and has made the interesting speculation that this sphingomyelin may normally originate from gangliosides localized in ganglion cells If it can be proved a dis turbance of such interconversion would be compatible with the changes found in AFI

## LIPID CONTENT OF THE BRAIN IN AMAUROTIC FAMILY IDIOCY

Gangliosides In 1939 Klenk reported his historic finding that the neuraminic acid content of gray matter was greatly increased (at least ten to twenty times normal) in infantile AFI [7 8] This finding was confirmed by him in several subsequent cases [12 95] in which it was also observed that the neuraminic acid (ganglioside) content of the cerebellar gray and of the dentate nucleus was quite high Gangliosides were also detectable *in appreciable quantities in the white matter* In his original report Klenk also included analyses from five cases of juvenile AFI in which the total brain content of gangliosides was not increased He peculated that these findings may have been due to more diffuse disease in these cases Other workers have confirmed the gan glioside increa e in infantile ca es (Tables 17 1 and 17 3) and Cumings

in total lipid content especially notable in the content of certain lipids Some of the earlier studies of changes in lipids during development have been summarized by Page [83] Among the most complete of recent studies are those of Brante in rats and human beings [84] Edgar in rabbits [84a] and of Folch Pi [85] and Uzman and Rumley [85a] in the mouse

TABLE 17 2 QUANTITATIVE SUMMARY OF LIPIDS IN BRAIN (GRAMS PER 100 GM FRESH TISSUE)

| *Lipids* | *Whole brain* | *Gray matter* | *White matter* |
|---|---|---|---|
| Total lipids | 10 4 | 5 0–6 2 | 16 0–22 0 |
| Total phospholipids | | 3 1–4 6 | 6 2–9 3 |
| Phosphatidyl cholines | | 0 6–1 5 | 0 9–1 9 |
| Phosphatidyl serines (calc as stearyl-oleyl) | 1 3–1 7 | 0 6 | 1 4 |
| Phosphatidyl ethanolamines (calc as stearyl-oleyl) | 2 3–2 6 | | |
| Phosphosphingosides (sphingomyelin + cephalin B ) | | 0 3–0 9 | 1 8–4 3 |
| Glycosphingosides (cerebrosides) | | 0 3–1 9 | 4 1–7 4 |
| Aldehydes in acetal phosphatides (as equimolar palmital and stearal) | 0 35 | 0 14 | 0 63–0 75 |
| Inositol phosphatides (inositol × 4 7) | 0 19 | | |
| Strandin and gangliosides | | 0 6–0 7 | 0 06–0 07 |
| Sulfatides | | | 0 9–1 2 |
| Phosphatidopeptides | | | 0 1 |
| Total nonsaponifiable material | | 1 9–2 3 | 6 0–8 3 |
| Cholesterol | | 0 6–1 4 | 3 6–5 4 |
| Hydrocarbons | 0 01 | | |

SOURCE F N LeBaron et al [8 ] reprinted with permission of The American Physiological Society

In the brain the neurons have reached their maximum number before birth [86] and the major increase in mass of the brain postnatally is in the white matter accompanying the myelination of the axons emanating from the ganglion cells Although other lipids especially phosphatides and cholesterol are present at birth in the gray matter the gangliosides are the typical lipid of the ganglion cells They are present in distinctly higher concentration in the cerebral cortex and basal ganglia than in white matter

No gangliosides were detected in normal white matter by Klenk [12] Svennerholm [16] considers gangliosides to be normally present in normal white matter but in much lower concentration than in the cortex (Table 17 1) Using microanalytic techniques Robins Eydt and Smith found that in the monkey cerebellar cortex ganglioside was mainly restricted to the granular layer much less appearing in the molecular layer [87]

ever to expect the AFI brain to show changes in lipid content characteristic of demyelination [84 96] including a decrease in cerebroside and sphingomyelin

One unusual case morphologically resembling infantile AFI has been reported by Favarger and Wildi [97] in which both gangliosides and cerebrosides were increased in the brain

*Increased Gangliosides in Other Disease*

The accumulation of gangliosides in ganglion cells of the brain is not limited to AFI

An outstanding example is Pfaundler Hurler disease or gargoylism This is a familial disease associated with mental deficiency multiple and varying deformities of bone and cartilage hepatosplenomegaly and corneal opacities [1 98] The morphologic changes in the brain are very similar to those in AFI and the ganglioside content is increased [1 2] In addition there are relatively large amounts of ganglioside like lipids and free mucopolysaccharide with a high hexosamine content in the brain meninges liver spleen lymph nodes and other organs [1] There may be increased mucopolysaccharide in serum [1] Dorfman and Lorincz have reported an abnormally high urinary excretion of at least two acid mucopolysaccharides one of which has been identified as $\beta$ heparin [99] It is generally held that this disease is more related to a broad defect in mucopolysaccharide metabolism than to any specific defect in ganglioside metabolism [1 99] although an opposing view has been taken [100]

Increase in brain gangliosides (or sialic acid) has been reported in other diseases associated with demyelination These include diffuse sclerosis and multiple sclerosis [14] and Niemann Pick disease (Chap 18)

## LABORATORY FINDINGS

*Sialic Acid in Blood and Cerebrospinal Fluid*

Measurements of sialic acid in a variety of body fluids and tissues have been reported and changes in concentration or the normal distribution with proteins described in a number of diseases [63–65 101–105] Saifer Volk and Aronson [105] postulate that in AFI there may be a shift of the serum sialic acid from the albumin bound to the globulin bound form According to their data the total sialic acid is not increased They also report a lower percentage of protein bound sialic acid in the cerebrospinal fluid in AFI Their studies do not permit conclusions concerning a specific abnormality in AFI

No abnormalities in serum proteins have been found [106] A possible increase in low-density lipoproteins in some cases has been reported

has reported increases in juvenile and adult cases as well (Table 17 3) Folch Pi has also reported increased concentrations of strandin in one infantile case of AFI [53]

TABLE 17-3 LIPIDS IN BRAIN

| Lipids | Normal brain | | | |
|---|---|---|---|---|
| | Cerebral white | | Cerebral cortex | |
| | Adult | 1 week-old infant | Adult | 1 week-old infant |
| Total phospholipid | 6 3 | 2 77 | 3 85 | ? 5 |
| Sphingomyelin | 2 1 | 0 52 | 0 98 | 0 54 |
| Cholesterol esters | 0 08 | 0 | 0 | 0 0? |
| Cerebroside | 5 8 | 0 32 | 1 68 | 0 3 |
| Neuraminic acid† | 0 | 0 | 160 | 270 |
| Hexosamine† | 295 | 700 | | |
| (Water % | 72 9 | 87 7 | 82 2 | 90 4) |

| Brain in three cases of amaurotic family idiocy | Cerebral white | | | Cerebral cortex | | |
|---|---|---|---|---|---|---|
| (Age yr | 1 5 | 4 | 23 | 1 5 | 4 | 23) |
| Total phospholipid | 3 6 | 3 9 | 4 6? | 3 0 | 3 12 | 2 08 |
| Sphingomyelin | 0 36 | 1 25 | 1 57 | 0 45 | 0 8? | 0 ?6 |
| Cholesterol | 0 | 0 32 | 0 | 0 | 0 05 | 0 |
| Cerebroside* | 2 4 | 1 7 | 2 43 | 1 6? | 1 83 | 1 95 |
| Neuraminic acid† | 40 | 50 | 55 | 3?0 | ?96 | 360 |
| Hexosamine† | 8?7 | 422 | 352 | | | |
| (Water % | ?9 8 | 74 5 | 72 6 | 82 3 | 83 4 | 83 0) |

Results in grams per 100 gm fresh brain

† Results in milligrams per 100 gm dry brain

SOURCE J N Cumings [16] reprinted with permission of Blackwell Scientific Publications Oxford

**Other Lipids** There is less certainty concerning changes in content of other lipids in the AFI brain In his early cases Klenk found one case in which the content of glycerophosphatides was low [8] this was possibly an artifact In several cases [8] the total sphingomyelin and cerebroside content seems to have been low This is also true especially for the white matter in the data of Cumings (Table 17 3) Folch Pi and Lees also report decreases in all other brain lipids in infantile AFI [53] The available data cannot be corrected for the pathologic changes occurring in the total mass of the white or gray matter It is reasonable how

*Conversion of Gangliosides*

Klenk has recently expressed a theory [12] that the lipids typical of myelin i e sphingomyelin and cerebrosides may arise from gangliosides provided by the axon Presumably the conversion of ganglioside should occur by one of two mechanisms involving




cleavage of hydrophilic groups from ganglioside to leave either (1) cerebroside or (2) ceramide for synthesis of sphingomyelin For this type of reaction the ceramide in ganglioside should ideally be similar to that in cerebroside and sphingomyelin Klenk [12] has observed in brain that the ceramide fatty acid may be predominantly stearic in both gangliosides and sphingomyelin but this is in contrast to the longer-chain more unsaturated acids in cerebrosides The finding by Svennerholm [35] that gangliosides may also contain unsaturated acids in significant quantities leaves this issue unsettled and to be solved by future chromatographic analyses

The earlier age for maximum incorporation of galactose into gangliosides [78] is consistent with their having a precursor role but more elaborate studies involving specific activity measurements are essential for establishing a precursor product relationship between gangliosides and the other lipids It may be argued that synthesis of a less complex compound (cerebroside) via a more complex one (ganglioside) is an awkward route but it is possible for example that ceramide for synthesis of the myelin lipids might be made available to the axon (or glial cell) in the more hydrophilic ganglioside form The apparent increases in ganglioside content of white matter in AFI and in total brain in other demyelinating diseases are also consistent with a decreased conversion of gangliosides to other sphingolipids

In the absence of knowledge concerning ganglioside metabolism even speculation concerning the precise nature of the supposed enzymatic defect is limited The theory of Svennerholm [16] suggesting a normal loss of hexosamine from gangliosides and a possible failure of this mechanism in AFI has been previously mentioned Little is known about the oligosaccharidases or sialidases which act upon gangliosides Cleavage of sialic acid or neuraminic acid from mucoproteins by enzymes from microorganisms has been widely studied Earlier attempts to demonstrate activity against substrate glycolipids from brain or erythrocytes [12 111]

[107] but there are no comprehensive data supporting abnormal changes in the plasma lipids

### *Enzymes in Extracellular Fluid*

Increased activity of "aldolase" and glutamic-oxalacetic transaminase in serum and spinal fluid and also of "phosphohexose isomerase" in spinal fluid in AFI has been reported by Aronson et al [108] Many of these same changes were observed in other patients with skeletal muscle atrophy from various causes and were not considered specific for AFI by the authors [108] It is of interest that serum acid pho phatase does not appear to be elevated in contrast to Gaucher s disease and a rare case of Niemann Pick disease (Chaps 18 and 19)

### *Vacuolated Lymphocytes*

Vacuolization of lymphocytes in peripheral blood has been observed in both AFI and in Niemann Pick disease and there is now considerable literature relative to the possible value of lymphocytes in diagnosis and detection of heterozygous carriers of these traits Von Bagh and Hortling [109] first reported abnormal lymphocytes in full blown cases in Sweden and also found similar vacuolization of lymphocytes in 4 parents of 2 unrelated patients and in 6 of 7 normal siblings of a patient in one family [110] Further observations of this phenomenon have been reviewed by Spiegel Adolf et al [106] who also report that similar blood findings occur in infantile ca es of AFI In their studies both parents of 2 patients and a sibling of 1 had vacuolated lymphocytes but parents of several other patients did not They suggest that the longer the infantile patients survive the more likely vacuolization is to be observed

The explanation for this phenomenon is not known It suggests a possible means for detection of heterozygotes however and should be sought in more patients and their relatives until its value has been established The finding is not specific and care should be taken to eliminate other known causes of such changes in the lymphocytes [106]

## POSSIBLE PATHOGENESIS OF AFI

It is too early to propose a cogent theory of the pathogenesis of AFI It seems quite likely that the basic defect in this disease is the absence of an enzyme or some other mechanism resulting in the continued accumulation of gangliosides in the ganglion cells The presence of so much excess lipid causes the cells to enlarge grotesquely and eventually die As the cells die lipid spills out and is scavengered by the glial cells Demyelination or a failure of myelination if it has not already occurred takes place either because of the death of the cell or because formation of myelin lipid is impaired by the primary defect in ganglioside degradation [12]

among the Jewish population of the world The grand parents of their cases came largely from northeastern Europe

Slome found the consanguinity rates for all racial groups were abnormally high Roughly 25 per cent of the Jewish parents and 50 per cent of the Gentiles were consanguineous Of these two groups about half the parental relationships were as close as first cousins

### JUVENILE AFI

Although the morphologic changes in infantile and juvenile AFI are similar and the differences in ganglioside increase could be a matter of degree genetic studies suggest that they are not necessarily different expressions of the same mutation There is no tendency for the infantile and juvenile cases to occur within the same families The disease has been observed widely in Caucasian peoples notably in Scandinavia and in African and Asiatic races Jewish cases are extremely rare if they have been reported at all [*6*] Sjogren [*113*] analyzed 59 families in which 115 cases of juvenile AFI occurred and concluded that the Scandinavian strain he was studying was also inherited as an autosomal recessive trait As with the infantile form the juvenile type involves the sexes equally Rayner has calculated that the average morbidity in Sweden of juvenile AFI may be 1 40 000 live births His calculations include the approximation that heterozygotes must occur in that country at a rate of about 1 1 000 [*110*]

The possible value of vacuolization of lymphocytes in search for heterozygous ' carriers of the AFI gene or genes has been mentioned above

The recently published studies of van Bogaert and Klein [*27*] contain personal observations of 11 families involved with infantile juvenile or adult cases of amaurotic idiocy The pedigrees affected with the infantile variety include the interesting family studied successively by Falkenheim and then by R and L Rochling in which 13 cases occur in 3 generations Their pedigrees also indicate that consanguinity is common in affected families They include an interesting example of double consanguinity within a large Jewish pedigree the disease occurring only in the progeny of these doubly related matings In non Jewish pedigrees consanguinity was usually present

A complicated and interesting pedigree presented by van Bogaert and Klein [*27*] contains the only report of the occurrence of Niemann Pick disease and infantile AFI in the same family Unfortunately chemical studies were inadequate to establish the diagnosis with certainty or to exclude the possible presence of a new disease with morphologic and clinical features combining those of classical Niemann Pick disease and AFI

Late or adult forms of amaurotic idiocy appear to be familial and it

were unsuccessful Rosenberg and Chargaff have recently demonstrated however that sialic acid can be split from normal human brain mucolipid by the *Vibrio cholera* sialidase [59] It is most interesting that sialic acid was not hydrolyzed from mucolipid isolated from an infantile AFI brain under the same conditions [59]

*Abnormal Gangliosides*

The highly polymerized ganglioside or mucolipid isolated by Rosenberg and Chargaff from the AFI brain appeared to have some different properties from the normal mucolipid as was described above They have raised the speculation that the absence of the usual peptide moiety in the AFI mucolipid may have been related to its failure to serve as a substrate for sialidase Obviously these speculations are of great interest and must await much more research on the purity and physiologic significance of these large lipid aggregates which may be isolated from brain There is no evidence so far that the chemical components of gangliosides such as the ceramide or hexose moieties, are dissimilar in the normal and AFI brains but the analyses have also not been sufficiently quantitative to prove this conclusively

Thus the defect responsible for infantile AFI is far from clarified There is also no certainty that the same defect or trait is responsible for all cases diagnosed as AFI by either the clinician or the analytical chemist If the defect should be the same presumably in the older children or adults the enzymatic lack (?) is incomplete or partially compensated resulting in less cell death and demyelination

## GENETICS

### INFANTILE AFI

The existing data support the inheritance of infantile AFI as an autosomal recessive trait The most comprehensive study is that of Slome [112] who carefully analyzed 135 cases reported up to 1934 The observed frequency of cases in 69 sibships half of which contained four or more members was reasonably close to 25 per cent the portion predicted on the assumption that each parent carried a single recessive gene with expression of the disease only in the homozygous progeny There was no sexual predilection Aronson et al [21a] have recently analyzed 122 cases with very similar conclusions The uniformly semilethal character of the infantile form accounts for the failure to observe direct transmission from one generation to the next

The disease is much more common in Jews In Slome s series approximately 20 per cent of cases were in non Jews In the series of Aron on et al [21a] the percentage was even lower They also emphasize the indications of an inhomogeneous distribution of the abnormal gene

10 Klenk E Über die Ganglioside des Gehirns b i der infantilen amaurotischen Idiotie von Typus Tay Sa hs Bir deutsch cl em Gesell ch 75 163 194?
11 Kl nk I Über die Canglio i k eine neue C ruppe von zuckerhaltingen Gehirn lipoiden Zt chr phy iol Chem 273 6 194?
12 Klenk E The pathological chemi try of the leveloping brain in *Biochemistry of the Developing Nerro s Syste* i e lite l by H Waelsch p 3?7 Academic Pre s Inc New York 1?55
13 Cumings J N The cerel ral lipi ls in disseminated sclero is and in amaurotic family idiocy Brain 76 551 1953
14 Cumings J N Lipid chemistry of the brain in demyel nating di eases Brain 78 554 1955
15 Cumings J N The diagnostic value of lipi l estimations in the cerebral lip do es in *Ce bral Lip dos s* edited by I van Bogaert J N Cumings and A Lowenthal p 11? Blackwell Oxford 195
16 Svennerholm L The nature of the ganglio ides in Tay Sach di ease in *Cerebral L p doses* edited l y L van Bogaert J N Cumings and A Lowenthal p 139 Blackwell O for l 1957
17 Tay W *Symmetrical changes in the region of the yellow spot in each eye of an* infant Tr Ophth Soc U Kingdom 1 155 1881 A third instance in the same fam ly of symmetrical changes in tl e region of the yellow spot in each eye of an infant closely resembling tho e of embolism ibi l 4 158 1884
18 Sachs B On arre ted cerebral development with special reference to its cort cal pathology J Nerv & Ment Dis 14 541 188
19 Sachs B A family form of i liocy generally fatal associated with early l lind ness J Nerv & Ment Di 21 475 1896
0 Sachs B and Hausman I *Nervous and Me tal Di rders f om Bi th th ough Adol scence* Hoeber New York 1? 6
21 Kanof A Aronson S M and Volk B W Cl nical progression of amaurotic family idiocy A M A J Dis Child 97 656 195?
1a Aronson S M Aronson B F and Volk B W A genetic p ofile of infantile amaurotic family idio y A M A J Dis Chil l 98 50 1959
2? Rothstein J L an l Welt S Infantile amaurotic family idiocy Am J Dis Chil l 62 801 1941
?3 Glol is J H Amaurotic family i liocy J Mt Sinai Hosp 9 451 194_
4 Aronson S M Volk B W and Epstein N Morphologic evolution of amauro ti family idiocy the protracted phase of the di ease A M A J Dis Child 97 684 195?
25 Jaffe R H The reticuloendothelial system in *Handbook of Hematology* ed ted by H Downey vol ? sec 15 Hoel er New York 1938
?6 Pick L an l Biel chow ky M Über lipoidzell ge Splenomegalie (Typu Niemann Pick) un l amauroti che I l otie Kl n Wchnschr 6 1631 19?7
Van Bog ært L an l Klein D Ol servations sur l hérédité des d ot es amauro tiques et de la pl?no hépatomégalie lipid enne (11 familles) J g?nét hum 4 3 19 5
28 Norman R M and Wool N A congenital form of amau otic family idiocy J Neurol Psychiat 4 175 1941
?9 Bro n N J Carver B D and Dodgson M C H A second case in the same family of congenital f n lial cereb al l p do re embling amaurot c family id o y Arch Dis Chil lhoo l 29 48 195?
30 Ha m ( B and Parmelee A H Amau otic family idio y (Tay-Sachs type) a ca e with a protracted course Am J Dis Ch ld 35 87 19 8
31 Greenfield J G The retina in cerebro pinal l p do is Proc Roy Soc Med (London) 44 68? 19?1
3 Goldste n I and Wexl r D Niemann Pick s di ea e with cherry red spots in the macula ocular pathology Arch Ophth (Ch ago) 5 704 1931
33 D ezel l B Histocl emisel e Ui tersu hungen am primaren Lipoido en Am aurotische Idiotie Gargoyli mus N emai n l icks he Krankl eit Gauchersche Krankhe t mit besonderer Berucks cht gung des Zentralnervensystems Virch Arch path Anat 326 89 195?

has been postulated that the inheritance is recessive [27] but sufficient cases have not been studied to establish the mode with certainty

## SUMMARY

1 Amaurotic family idiocy (AFI) is a sphingolipidosis characterized by dementia paralysis and blindness The infantile form also known as Tay Sachs disease begins within the first year of life and is fatal by the third Symptoms may appear later, a juvenile form which usually becomes manifest at age 5 to 10 has also been extensively studied

2 In all forms of AFI the characteristic pathologic alterations are restricted to the nervous system In the infantile form it has been conclusively shown that the ganglion cells are overloaded with gangliosides which are hydrophilic lipids containing sphingosine fatty acid hexose hexosamine and sialic acid The eventual death of the ganglion cells is accompanied by demyelination or failure of myelination and an increase in lipid laden glial cells

3 The complete sequence of steps in the synthesis of gangliosides is not understood, and little is known of the pathways of degradation or the possible physical state of the gangliosides in brain It is therefore not possible to determine what enzymatic defects, if any, are responsible for AFI

4 The infantile and juvenile forms of AFI both appear to be inherited as autosomal recessive traits The genes have a different racial distribution however and it is not known whether they represent different manifestations of the same basic disease Vacuolization of lymphocytes in peripheral blood has been observed in some probands and their relatives It has been suggested that this may provide a means of determining heterozygotes

## BIBLIOGRAPHY


1 Brante G Chemical pathology in gargoylism in *Cerebral Lipidoses* edited by L van Bogaert J N Cumings and A Lowenthal p 164 Blackwell Oxford 1957
2 Uzman L L Chemical nature of the storage substance in gargoylism Hurler Pfaundler s disease A M A Arch Path 60 308 1955
3 Fick L Über die lipoidzellige Splenohepatomegalie Typus Niemann Pick als Stoffwechselerkrankung Med Klin (Munich) 23 1483 1927
4 Thudichum J L W *Die chemische Konstitution des Gehirns des Menschen und der Tiere* F Pietzcker Tubingen 1901
5 Jervis G A Familial idiocy due to neuronal lipidosis (the so-called late amaurotic idiocy) Am J Psychiat 107 403 1950
6 Jervis G A Juvenile amaurotic idiocy A M A J Dis Child 97 663 1959
7 Klenk E Beitrage zur Chemie der Lipoidosen Niemann Picksche Krankheit und amaurotische Idiotie Ztschr physiol Chem 262 128 1939-1940
8 Klenk E Beitrage zur Chemie der Lipoidosen Ztschr physiol Chem 267 1 8 1940
9 Klenk E Neuraminsaure das Spaltprodukt eines neuen Gehirnlipoids Ztschr physiol Chem 268 50 1941

Submaxillarismucins und Harnmucoproteids Die Isolierung von Neuraminsaure als Spaltprodukt Ztschr physiol Chem 291 147 1952
62 Blix F G Gottschalk A and Klenk L Proposed nomenclature in the field of neuraminic and sialic acids Nature London 179 1088 1957
63 Gottschalk A Neuraminic acid the functional group of some biologically active mucoproteins Yale J Biol & Med 28 525 1956
64 Cornforth J W Firth M E and Gottschalk A The synthesis of N acetyl neuraminic acid Biochem J 68 57 1958
65 Warren L Sialic acid in human semen and in the male genital tract J Clin Invest 38 755 1959
66 Warren L The thiobarbituric acid assay of sialic acids J Biol Chem 234 1971 1959
67 Brady R O and Koval G J The enzymatic synthesis of sphingosine J Biol Chem 233 26 1958
68 Brady R O Formica J V and Koval G J The enzymatic synthesis of sphingosine II Further studies on the mechanism of the reaction J Biol Chem 233 1072 1958
69 Comb D G and Roseman S Composition and enzymatic synthesis of N acetylneuraminic acid (sialic acid) J Am Chem Soc 80 497 1958
70 Zabin I and Mead J Biosynthesis of sphingosine II The utilization of methyl labeled acetate formate and ethanolamine J Biol Chem 211 87 1954
71 Sprinson D B and Coulon A The precursors of sphingosine in brain tissue J Biol Chem 207 585 1954
72 Sribney M and Kennedy E P The enzymatic synthesis of sphingomyelin J Biol Chem 233 1315 1958
73 Zabin I Biosynthesis of ceramide by rat brain homogenates J Am Chem Soc 79 5834 1957
74 Thannhauser S J and Reichel M Studies on animal lipids X The nature of cerebrosidase Its relation to the splitting of polydiaminophosphatide by poly diaminophosphatase J Biol Chem 113 311 1936
75 Sribney M and Kennedy E P The enzymatic synthesis of sphingomyelin J Am Chem Soc 79 5325 1957
76 Burton R M Sodd M A and Brady R O The incorporation of galactose into galactolipides J Biol Chem 233 1053 1958
77 Radin N S Martin F B and Brown J R Galactolipide metabolism J Biol Chem 224 499 1957
78 Mandelstam P and Burton R M Incorporation of glucose and galactose into glycolipids of rat brain tissue Fed Proc 18 280 1959
79 Maxwell E S Kalckar H M and Burton R M Galacto-waldenase and the enzymic incorporation of galactose 1 phosphate in mammalian tissues Biochim et biophys acta 18 444 1955
80 McIlwain H *Biochemistry and the Central Nervous System* Little Brown Boston 1955
81 Yamakawa T and Suzuki S The chemistry of the lipids of post hemolytic residue or stroma of erythrocytes I Concerning the ether-soluble lipids of lyophilized horse blood stroma J Biochem (Japan) 38 199 1951
82 LeBaron F N and Folch J Structure of brain tissue lipides Physiol Rev 37 539 1957
83 Page I H *Chemistry of the Brain* Charles C Thomas Springfield Ill 1937
84 Brante G Studies on lipids in the nervous system with special reference to quantitative determination and topical distribution Acta phys ol scandinav 18 Suppl 63 1 1949
84a Folch J W F Myelination studied by quantitative determination of myelin lipid ... Acta a at 31 451 19..
85 Folch Pi J Composition of the brain in relation to maturation in *Biochemistry of the Developing Nervous System* edited by H Waelsch p 121 Academic Press Inc New York 1955
85a Uzman L L and Rumley M K Changes in the composition of the developing mouse brain during early myelination J Neurochem 3 170 1958

34 Diezel P B Histochemical study of primary lipidoses in *Cerebral Lipidoses* edited by L van Bogaert J N Cumings and A Lowenthal p 11 Blackwell, Oxford 1957
35 Svennerholm L Determination of gangliosides in nervous tissue in *Cerebral Lipidoses* edited by L van Bogaert J N Cumings and A Lowenthal p 1?? Blackwell Oxford 1957
36 Landing B H and Freiman D G Histochemical studies on the cerebral lipidoses and other cellular metabolic disorders Am J Path 33 1 1957
37 Davison C Disturbances in lipoid metabolism and the central nervous system J Mt Sinai Hosp 9 389 1942
38 Drabkin D L *Thudichum Chemist of the Brain* University of Pennsylvania Press Philadelphia 1958
39 Carter H E Glick F J Norris W P and Phillips G E Biochemistry of the sphingolipides III Structure of sphingosine J Biol Chem 170 285 194?
40 Carter H E Sphingolipides in *Chemistry of Lipides as Related to Atherosclerosis* edited by I H Page p 82 Charles C Thomas Springfield Ill 1958
41 Carter H E and Shapiro D Configuration of dihydrosphingosine J Am Chem Soc 75 5131 1954
42 Sweeley C C A gas chromatographic method for sphingosine assay Biochim et biophys acta 36 268 1959
43 Thannhauser S J and Frankel E Über das Lignocerylsphingosine Ztschr physiol Chem 203 183 1931
44 Fränkel E and Bielschowsky F Untersuchungen über die Lipoide der Säugetierleber II Über das Vorkommen des Lignocerylsphingosins in der Schweineleber Ztsch physiol Chem 213 58 1932
45 Tropp C and Wiedersheim V Untersuchungen über Lipoide tierischer Organe VIII Über das Vorkommen des Lignocerylsphingosin in der Rindermilz Ztschr physiol Chem 222 39 1933
46 Klenk E and Rennkamp F Über die Ganglioside und Cerebroside der Rindermilz Ztschr physiol Chem 273 253 1942
47 Klenk E and Lauenstein K Über die Glykolipoide und Sphingomyeline des Stromas der Pferdeerythrocyten Ztschr physiol Chem 295 164 1953
48 Klenk E On gangliosides A M A Am J Dis Child 97 711 1959
49 Walz E Über das Vorkommen von Kerasin in der normalen Rindermilz Ztschr physiol Chem 166 210 1927
50 Levene P A and Landsteiner K On some new lipoids J Biol Chem 75 607 1927
51 Blix G Einige Beobachtungen uber eine hexosaminhaltige Substanz in der Protagon fraktion des Gehirns Skandinav arch physiol 80 46 1938
52 Blix G Svennerholm L and Werner I The isolation of chondrosamine from gangliosides and from submaxillary mucin Acta chem scandinav 6 358 1952
53 Folch Pi J and Lees M Studies on the brain ganglioside strandin in normal brain and in Tay Sachs disease A M A J Dis Child 97 730 1959
54 Bogoch S Studies on the structure of brain ganglioside Biochem J 68 319 1958
55 Folch Pi J Arsove S and Meath J S Isolation of brain strandin a new type of large molecule tissue component J Biol Chem 191 819 1951
56 Rosenberg A Howe C and Chargaff E Inhibition of influenza virus haemagglutination by a brain lipid fraction Nature London 177 234 1956
57 Rosenberg A and Chargaff E A study of a mucolipid of ox brain J Biol Chem 230 1031 1958
58 Rosenberg A and Chargaff E Nitrogenous constituents of an ox brain mucolipid Biochim et biophys acta 21 588 1956
59 Rosenberg A and Chargaff E Some observations on the mucolipids of normal and Tay-Sachs disease brain tissue A M A J Dis Child 97 739 1959
60 Blix G Über die Kohlenhydratgruppen des Submaxillarismucins Ztschr physiol Chem 240 43 1936
61 Klenk E and Lauenstein K Zur Kenntnis der Kohlenhydratgruppen des

109 von Bagh K and Hortling H Blodfynd vid juvenil amaurotisk idioti Nord med 38 1072 1948
110 Rayner S Juvenile amaurotic idiocy diagnosis of heterozygote Acta genet 3 1 1952
111 Faillard H Über die Abspaltung von N acetylneuraminsäure aus Mucinen durch das Receptor Destroying Enzyme aus *Vibrio cholerae* Ztschr physiol Chem 307 62 1957
112 Slome D The genetic basis of amaurotic family idiocy J Genet 27 363 1934
113 Sjögren T Die juvenile amaurotische Idiotie Hereditas 14 197 1931

86 Richter D The metabolism of the developing brain in *Biochemistry of the Developing Nervous System* edited by H Waelsch p 2 5 Academic Press Inc New York 1955
87 Robins L Eydt K M and Smith D E *Distribution of lipides in the cerebellar cortex and its subjacent white matter* J Biol Chem 220 677, 1956
88 Debuch H and Stammler A Über die Lipoide der Ganglienzellkerne aus der Rinde des Groshirns und des Kleinhirns Ztschr physiol Chem 305 111 1956
89 Johnson A C McNabb A R and Rossiter R J Lipids of normal brain Biochem J 43 573 1948
90 Johnson A C McNabb A R and Rossiter R J Concentration of lipids in the brain of infants and adults Biochem J 44 494 1949
91 Koch W and Koch M S Contributions to the chemical differentiation of the central nervous system III The chemical differentiation of the brain of the albino rat during growth J Biol Chem 15 423 1913
92 Langworthy O R Development of behavior patterns and myelinization of the nervous system in the human fetus and infant in *Contributions to Embryology* vol 24 No 139 Carnegie Institution of Washington D C 1933
93 Finean J B and Robertson J D Lipids and the structure of myelin Brit M Bull 14 267 1958
94 Glees P *Neuroglia Morphology and Function* Charles C Thomas Springfield Ill 1955
95 Klenk E Über die Verteilung der Neuraminsäure im Gehirn bei der familiären amaurotischen Idiotie und bei der Niemann Pickschen Krankheit Ztschr physiol Chem 282 84 1947
96 Johnson A C McNabb A R and Rossiter R J Chemical studies of peripheral nerve during Wallerian degeneration Biochem J 45 500 1949
97 Favarger P and Wildi E Chemical analysis and histochemical examination of an atypical case of Tay Sachs disease in *Cerebral Lipidoses* edited by L van Bogaert J N Cumings and A Lowenthal p 146 Blackwell Oxford 1957
98 Jervis G A Gargoylism (lipochondrodystrophy) study of 10 cases with emphasis on formes frustes of the disease Arch Neurol & Psychiat 63 687 1950
99 Dorfman A and Lorincz A E Occurrence of urinary acid mucopolysaccharides in the Hurler syndrome Proc Nat Acad Sc (U S) 43 443 1957
100 Seitelberger F The position of gargoylism amongst the sphingolipidoses on the basis of the histochemistry of cellular storage substances in *Cerebral Lipidoses* edited by L van Bogaert J N Cumings and A Lowenthal p 77 Blackwel Oxford 1957
101 Bohm P and Baumeister L Über das Vorkommen neuraminsaurehaltiger Glykoproteide in Korperflussigkeiten Ztschr physiol Chem 305 42 1956
102 Uzman L and Rumley M K Neuraminic acid as a constituent of human cerebrospinal fluid Proc Soc Exper Biol & Med 93 497 1956
103 Bogoch S Cerebrospinal fluid neuraminic acid deficiency in schizophrenia a preliminary report Am J Psychiat 114 172 1957
104 Ross J and Bohm P Neuraminsaurehaltige Glykoproteide des Liquor cerebrospinalis bei Erkrankungen des Nervensystems Klin Wchnschr 35 351 1957
105 Saifer A Volk B W and Aronson S M Neuraminic (sialic) acid studies of biological fluids in amaurotic family idiocy and related disorders A M A J Dis Child 97 745 1959
106 Spiegel Adolf M Baird H W Kollia D and Szekely E G Cerebrospinal fluid serum and blood investigations in amaurotic family idiocy A M A J Dis Child 97 676 1959
107 Barclay M Calathes D N and Dargeon H W Plasma lipoproteins in the lipidoses A M A J Dis Child 97 713 1959
108 Aronson S M Saifer A and Volk B W Serial enzyme studies of serum and cerebrospinal fluid in amaurotic family idiocy A M A J Dis Child 97 684 1959

the years 1922–1927 Ludwick Pick correlated these cases into a single entity distinct from Gaucher s disease on anatomic grounds and called the new syndrome lipoid cell splenomegaly [5 6] The disease rapidly became known by the term most familiar today Niemann Pick disease

In the early cases reported as Gaucher s disease Wahl and Richardson [7] and Siegmund [3] found increased lipid phosphorus and cholesterol in the liver and spleen Further studies by Bloom and Kern [8] and a number of other workers [9–13] established that an increase in phospholipid was indeed characteristic of Niemann Pick tissues In 1934–1935 Klenk made the important discovery that the elevated lipid phosphatide in the spleen [14] liver and brain [15] was predominantly sphingomyelin This was quickly confirmed by others [16–20]

About 100 cases have been reported since Niemann s first case Videbaek analyzed 73 of these in 1949 and reported for the first time a patient surviving beyond the third year [21] Crocker and Farber have recently added 18 cases personally observed by them including others detected in infancy which have had a protracted course [22] Several cases compatible with Niemann Pick disease in adults have been diagnosed at autopsy The first of these were in two Swiss brothers who died at the ages of 29 and 33 [23 24] In a 51 year old male studied by Terry et al [25] there were tissue lipid changes comparable to those seen in some infantile patients [22]

As time progresses more cases of Niemann Pick disease surviving infancy may be observed [1] An interesting case has been called to the author s attention by Dr Frank L Miller [26] This is a 42 year-old soldier completely asymptomatic who was found by x ray to have an isolated pulmonary lesion Biopsy specimens of lung rib and liver contained histologic and chemical changes compatible with the diagnosis

A number of reviews of Niemann Pick disease have appeared The most recent and comprehensive is that of Crocker and Farber [22] Others include those of Thannhauser [27] Pfandler [24 28] Videbaek [21] Baumann et al [29] Canmann [30] and Maurer [31] The most impressive histologic studies are those made by Bloom [32]

## CLINICOPATHOLOGIC CORRELATIONS

The pathologic changes in Niemann Pick disease include (1) the accumulation of lipid in reticuloendothelial cells sometimes everywhere

[1] The author has studied a 19-year-old girl through the courtesy of Drs Milton Shy and Andrew Engle of the National Institute for Neurological Diseases and Blindness This patient was found to have splenomegaly at age 4 and progressive neurologic changes beginning at age 1[illegible] She is able to live a reasonably normal life at present In this case a presumptive diagnosis of Niemann Pick disease has been made by bone marrow biopsy only

# Chapter 18

## Niemann Pick Disease

---

*Donald S Fredrickson*

### DEFINITION

Niemann Pick disease (sphingomyelin lipidosis) is a relatively rare condition characterized by widespread accumulation of lipid mainly sphingomyelin in reticuloendothelial nervous and other tissues The usual clinical manifestations include hepatosplenomegaly retarded physical and mental growth and neurologic disorders which may often include a cherry red spot in the retina These most commonly develop within the first year of life and progress to a fatal issue by the third year The disease may occasionally develop later and progress much more slowly The condition is inheritable but the biochemical basis is presently unknown

### HISTORICAL ASPECTS

In 1914 Niemann a Berlin pediatrician observed a female infant born of Polish Jewish parents who died at 18 months of a severe malady characterized by feeding problems abdominal distention hepatosplenomegaly facial pigmentation and edema [1] At autopsy striking yellow white lesions were found in the spleen liver, abdominal lymph nodes kidneys and adrenal glands The yellow color was due to masses of large cells which stained with Sudan III and which Niemann thought were consistent with the cells previously described in Gaucher s di ease He considered the early age of development and the rapid malignant cour e in his patient atypical of the available descriptions of the latter disease however, and chose to report his case as *ein unbekanntes Krankheitsbild*

Knox Wahl and Schmeisser [2] and Siegmund [3] shortly thereafter reported very similar cases but under the diagnosis of Gaucher s di ease Mandlebaum and Downey [4] strongly disputed the diagnosis in one of these cases [2] but they failed to associate it with Niemann s case During

It is generally accepted from the work of Bloom and of Pick [6 32] that the NP cells arise from the accumulation of lipid by reticulum cells in lymphoid tissue the resting wandering cells of the connective tissue and perivascular embryonic cells and phagocytes all over the body The endothelial cells in the liver are also potential foam cells but probably not the endothelial cells in the peripheral blood vessels [32]

Supravital preparations of biopsy material from bone marrow or other tissue often afford the best means of detecting the presence of the NP cells They are easily distinguished from the Gaucher cells (Fig 19 1) which have a very different and characteristic pattern in the cytoplasm The morphology of the NP cell is not characteristic of sphingomyelin accumulation however since identically appearing cells may be found in the tissues of patients with severe hyperlipidemia (Chap 16) and have been observed in patients with cephalin lipidosis [33] (see below) There is no absolutely specific histologic stain for sphingomyelin The Smith Dietrich reaction which is presumed to stain unsaturated fatty acids and hence phospholipids usually stains the NP cells a dark blue or black A positive reaction is helpful but the degree of staining is variable Other staining reactions of the cells are described in detail elsewhere [34 35] A histologic diagnosis should be considered presumptive and corroborated whenever possible with tissue chemical studies

## SPLEEN LYMPHOID TISSUE AND BONE MARROW

No doubt because of their high content of reticulum cells these tissues are among the earliest involved in detectable foam (NP) cell accumulation The spleen increases in size most rapidly between 1 and 2 years of age and may become tenfold enlarged [32] The splenic pulp may be completely replaced by sheets of foam cells

The bone marrow likewise contains many foam cells some free others still in the reticular syncytium [32] Despite such dramatic changes abnormalities in the peripheral blood are not at all remarkable with the exception of vacuolization of agranulocytes A moderate usually microcytic anemia is common The leukocyte count varies but not in any characteristic manner Platelets may occasionally be decreased but the megakaryocytes appear normal If splenectomy is eventually performed leukocytosis and thrombocytosis promptly occur postoperatively [22]

In nearly all cases careful search will reveal discrete vacuoles in lymphocytes and monocytes in smears of peripheral blood or bone marrow The cells have an appearance identical to that of the vacuolated leukocyte seen in patients with amaurotic family idiocy It is reasonable to presume the vacuoles are formed by accumulation of the offending lipid in these diseases Attempts to demonstrate an increased lipid content of the leukocytes (buffy coat) in blood from patients with Niemann Pick disease have not been successful [22]

Lymphoid tissues including the thymus commonly contain cyto-

in the body and in parenchymal cells of many organs and (2) patchy destruction of ganglion cells and demyelination throughout the nervous system This pervasive involvement gives rise to a complex but fairly predictable array of symptoms and signs which classically appear in the first 6 months of life and gradually progress

The first indications of abnormality usually include abdominal enlargement poor feeding patterns and delays in growth and development The changes which occur in the usual 2 to 3 year interval between onset and death may perhaps be understood most clearly through a systematic correlation of the anatomic and clinical findings

### THE NIEMANN PICK (NP) CELL

The histologic trademark of the disease is the Niemann Pick cell or large reticuloendothelial cell (20 to 90 microns in diameter) shown in Fig 18-1 The cytoplasm is filled with many droplets or particles which

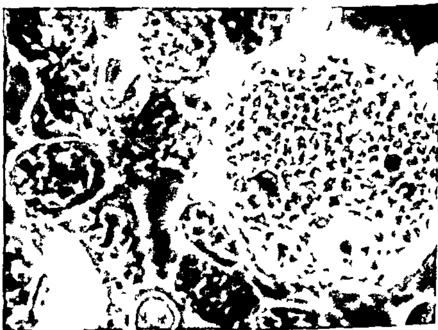


Fig 18 1 The Niemann Pick cell photographed through the phase microscope in a supravital preparation of bone marrow aspirate The size of the cell may be compared with that of the adjacent erythrocytes (*Courtesy of Dr Georg Brecher National Institutes of Health*)

are usually fairly uniform in size and which give the cell a foamy or mulberry appearance Occasionally only a few large vacuoles are present, a single nucleus is common but up to 18 have been described [27] Bloom has discussed the morphology of these cells in most elaborate detail [*32*]

parietal fissures [37] With rare exception [38] the ventricles are not dilated and the over all weight of the brain is not increased [22] Occasional patients do have prominent frontal bossae and wide fontanelles commonly seen in the older children with amaurotic family idiocy (AI I) when extensive gliosis has increased the size of the brain Morphologically the changes are very similar to those described in the latter disease (Chap 17) The ganglion cells are swollen with pale cytoplasm which is often frankly vacuolated There are granular degeneration of the Nissl substance swelling of dendrites and disappearance of the normal fibrillae The white matter is often decreased in volume with a decrease in the normal staining reactions for myelin but without other remarkable microscopic changes [22] Foam cells derived from lipid laden glial cells may appear to replace much of the substance of the brain and are also prominent in the leptomeninges tela choroidea and the connective tissue about the cerebral vessels [6 37 39]

In addition to the brain similar changes occur in the spinal cord the autonomic nuclei and those cells of the adrenal medulla related to the sympathetic nervous system Large vacuoles have been described in the sheaths of medullated nerves presumably because of accumulation of lipid

Signs of nervous system dysfunction are considered to initiate the late stages of the disease [27] and are usually progressive in development From the degree of anatomic involvement it is understandable that all types of abnormalities in psychic motor and sensory function may occur In the earliest months of life poor feeding habits may be a first sign followed by an obvious lag in mental and motor development Then may follow muscular rigidity involuntary contractions asynergia athetosis tremors and seizures The cranial nerve nuclei are often involved Deafness is quite common because of nerve or central involvement or lesions in the ear itself [29 40]

Mental retardation becomes progressively more severe and has been present in all children before death Previous learning may disappear and the state of the patient may become almost vegetative In some cases developing later in childhood behavior and personality problems may be among the first symptoms [22] Two cases have been reported which are especially notable in that they have remained without detectable nervous system involvement after many years of known disease

The cerebrospinal fluid is normal Nonspecific electroencephalographic abnormalities have been recorded

## EYES

A cherry red spot has been found in the retina in 20 [22] to 60 [21] per cent of cases This phenomenon consists of a white or grayish macular area in the center of which the fovea appears as a red spot The lesion is similar to that seen in infantile AI I although some authors describe

architectural changes similar to those in the spleen The lymph nodes may be considerably enlarged

LIVER

The liver increases in size and undergoes color changes from reddish gray to yellow mainly because of conversion of both Kupffer and endothelial cells to foam cells The parenchymal cells may also appear vacuolated and may increase in size It has been suggested that changes in these latter cells may be due not to lipid accumulation but to collection of glycogen or some other degenerative process Diezel [36] on the other hand has reported that in one case the parenchymal cells were also filled with PAS negative granules which he interpreted as sphingomyelin The degree of distortion of the liver architecture varies greatly from case to case There is usually some increase in periportal connective tissue but "cirrhotic" changes are rarely as marked as they may be in Gauchers disease

In a few patients jaundice may appear early and persist for a few months [22] In at least one case [22] evidence has been obtained that this may be because of biliary obstruction produced by large lymph nodes near the hilum In the absence of jaundice the usual flocculation test results [21 22] and Bromsulfalein excretion on the rare occasion when it has been measured [22] are normal Glucose tolerance test results and serum protein concentration are also usually normal even in the most advanced cases

THORACIC VISCERA

Histologic changes in the lungs may be difficult to find or they may be spectacular in their extent On occasion the alveoli have been completely filled with NP cells with prominent involvement of the septums and the area about blood vessels The lumens of the pulmonary capillaries may contain large numbers of free foam cells Such changes often produce an x ray picture of diffuse mottling or miliary nodularity Occasionally localized infiltration is present Chronic bronchitis and bronchopneumonia are common and frequently the apparent cause of death

Foam cells may also appear in the heart interspersed among cardiac muscle fibers Associated changes in cardiac size or function are uncommon Edema of the lower extremities is commonly seen late in the disease and may be due mainly to embarrassment of venous return by the enlarged abdominal organs

NERVOUS SYSTEM

Throughout the nervous system there occur destructive changes in the ganglion cells demyelination proliferation of glial cells and their conversion to foam cells and extensive scarring and fibrosis The changes may be diffusely distributed [37] or very irregular [22]

The brain is usually hard or leathery with gaping sylvian and inter

compiled from analyses of tissues made by Crocker and Farber from their Case 7 [22] an infant who developed symptoms at 3½ months and died at 21 months Extensive analyses have also been presented by Thann hau er [27] Terry et al [25] and Klenk [14 15 50–53] a number of studies of individual organs have been made by others [3 7–13 16–20 29 54 55]

In tissues other than brain the analyses may be summarized as showing (1) an increase in the absolute amount of sphingomyelin (often ten to fortyfold) and in the relative contribution of this compound to the total

TABLE 18-1 CHEMICAL ANALYSES (IN GRAMS PER 100 GM FRE H WEIGHT) OF FRESH OR FROZEN TISSUE FROM A CASE OF NIEMANN PICK DISEASE

| *O gan* | *T t l l p d* | *Chol terol* | *Ph phol p d* | *Sph g myel* |
|---|---|---|---|---|
| Spl | 12 6 (2 3 5) | 2 7 (0 3–0 4) | 7 9 (1 2) | 4 6 (0 –0 9) |
| Lymph d | 6 9 (2 3 5) | 0 6 (0 3 0 4) | 5 1 (1 2) | 7 (0 –0 9) |
| L | 14 8 (3–4) | 0 8 (0 3–0 4) | 10 1 (1 5 5) | 6 6 (0 1–0 5) |
| L g | 16 5 (2 3 5) | 0 9 (0 3–0 4) | 1 1 (1 2) | 6 8 (0 1–0 4) |
| B | | | | |
| G y m tt | 6 9 (3 9 5 2) | 0 9 (0 6–0 7) | 4 3 ( 3 3 0) | 1 7 (0 4–0 6) |
| Wh t m tt | 8 7 (7 8 14 0) | 1 2 (1 4 3 1) | 4 4 (4 0 6 0) | 1 6 (1 0–1 8) |

S A C C k t l (th C 7) [22] d t mb d f m th T l l III–V d VII
Th r m l t t (n th se f b f m B t [22]) h w r th

phospholipid (2) an increase in cholesterol associated in most cases with a rise in free cholesterol and possibly a reduction in the absolute amount of e terified cholesterol [27] and (3) usually smaller but never theless definite increases in the non sphingosine-containing phospho lipids The latter have not been extensively fractionated although Chargaff [20] reported that the largest fraction was cephalin in the spleen from one case In the organs of another case Klenk [15] found no increase in cephalin and only a small increase in lecithin compared with an enormous increase in phingomyelin The content of glyceride reported has been variable the determination usually having been made by ub-traction of the eparately determined lipid fractions from the total lipid An increa e in unaccounted lipid pre umably glyceride is also suggested by more recent data [22] Not all possibility of a new and unexpected lipid component has been eliminated by the available analyses but in crea ed phingomyelin remains the dominant change

Klenk originally found the phingomyelin content of brain increa ed [15] In one ca e Thannhau er et al reported a decrease in phingomyelin in whole brain although there was an increa e in total pho pholipid [19] Some di agreement in total brain lipid content may be more apparent than real since uch analy es must reflect a resultant of differing changes in the gray and white matter

certain differences in size or coloration [41, 42] This sign is not necessarily present at birth and may be unilateral The pathologic changes in the retina in these two diseases have been described in detail by several authors [43–45]

Additional constitutional abnormalities attributed to dysfunction of appropriate nervous centers [29] include persistent sweating vomiting salivation and fever

## BONES

The conversion of masses of reticular cells in the bone marrow to foam cells might be expected to produce more evident changes in bones Widened medullary cavities occasionally seen at autopsy [30 46 47] are rarely detected by x ray however and the typical "Erlenmeyer flask deformity" seen in Gaucher's disease has not been reported Osteoporosis no doubt related to nutritional inadequacy and inactivity is common Bone age is usually normal [22] Serum calcium and phosphorus have been frequently measured and found within normal limits

## SKIN

Changes in the skin occur some of which may be related to the infiltration of foam cells in the underlying connective tissue Pigmentation often brownish yellow in color is common It is sometimes diffuse but at other times localized to discrete *café au lait* spots Dark bluish "mongol" spots have also been seen on the skin and oral mucosa [27 48] Suppurative lesions about the face associated with foam cell infiltration and eruptive and infiltrative xanthomas have also been described [22 31]

## OTHER ORGANS

Alterations have been found in every organ So many tissue cell types are loaded with lipid that Pick discouraged the use of the term "lipoid histiocytosis" which Bloom [49] had favored for the disease as too limiting Not all the anatomic changes in epithelial and other cells have expressed themselves in detectable abnormalities in function Thus large yellow adrenal glands variably involved in the medulla and cortex may be present without signs of adrenal insufficiency Other endocrine glands including the gonads thyroid and pituitary and exocrine glands such as the pancreas and salivary glands may likewise be burdened with lipid without evident functional disability Renal function is not grossly abnormal although foam cells are present in the kidney glomeruli and the tubular epithelium may appear abnormal

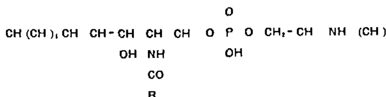
# CHEMICAL ABNORMALITIES

Niemann Pick disease is primarily a sphingomyelin lipidosis, but it is not exclusively so as the analyses in Table 18 1 indicate These data are

## SPHINGOMYELIN

### CHEMISTRY

Sphingomyelin consists of ceramide (N acylsphingosine) to the carbon 1 of which is attached phosphorylcholine [57–59]




The chemistry and biochemistry of sphingosine have been reviewed in Chap 17 The ceramide fatty acids in sphingomyelin characteristically include the 24 carbon acids lignoceric and nervonic but many other acids are undoubtedly present Klenk [16 53] and much later Bartsch [55] reported that in normal persons and in Niemann Pick patients the brain sphingomyelin contained mainly stearic acid and that the liver and spleen sphingomyelin contained palmitic stearic lignoceric and nervonic acids Slightly different proportions of the same acids were found in the sphingomyelin of these tissues by Thannhauser and Boncoddo [60] Chargaff has also analyzed the fatty acids of the sphingomyelin in Niemann Pick spleen [20]

It is important to note however that most of the published analyses have been based on determination of the melting points of the methyl esters of the fatty acids Gas-phase chromatography will greatly extend the available information concerning these components Analyses by this technique of the fatty acids present in plasma sphingomyelin in a case of Niemann Pick disease have been made by C C Sweeley (unpublished) At least 18 fatty acids were present the major acid was palmitic but the remainder varied from 12 to 24 carbons in length and included both saturated and unsaturated acids some of them uncommon and not readily identifiable Only such complete analyses will ultimately permit determination of whether or not the ceramide in Niemann Pick sphingolipids is normal From the earlier analyses it has been assumed that it probably is [27 53]

### OCCURRENCE

Sphingomyelin is widely distributed in the body The highest concentrations are found in the nervous system where this lipid is especially important in the myelin sheath The lipids of brain are discussed in more detail in Chap 17 In the myelin sheath of peripheral nerves the sphingomyelin concentration seems to be especially high [61] In Wallerian de-

Crocker and Farber separately analyzed gray and white matter and found the total lipid content of the white matter less than that of the gray a reversal of the usual composition This was interpreted as consistent with the deficiency in myelin suggested by the histologic findings In gray matter the concentrations of lipids were variable all components tending to be moderately elevated or on the high side of normal That the major change in brain is probably an increase in sphingomyelin has also been confirmed by Cumings [54] and Bartsch [55]

Klenk [52] found a slight increase in the ganglioside content of cerebral and cerebellar gray matter and in one case only in the white matter In none of the Niemann Pick cases did the increase in gangliosides in any part of the brain approach that found in infantile AI I Ganglio ides were not detectable in liver or spleen in either normal per ons or patients with Niemann Pick disease [52]

## LIPIDS IN EXTRACELLULAR FLUID

In some cases [11 21 29] in which serum lipids have been reported but by no means all [22] there has been a moderate hyperlipidemia Usually any increase has been in total cholesterol ' neutral fat or total fatty acids Occasionally the total phospholipid may be increased [11 27] In no case has markedly lactescent serum been reported In many full blown cases the concentrations of serum lipids and carotenoids may be normal [22 27] Most importantly the proportion of sphingomyelin to total serum phospholipids has been demonstrated repeatedly to be normal [27] The sphingomyelin content of whole blood has also been found to be within normal limits [20]

As with other tissues the lipids of extracellular fluid need to be examined more thoroughly by chromatographic methods The available analyses of the lipids in ascitic fluid and cerebrospinal fluid are within normal limits

## SERUM ACID PHOSPHATASE

In one 3 year old patient Hastrup and Videback [56] found a serum acid phosphatase of 22 Gutman units per 100 ml (normal 2 to 3 units) The pH optimum of this enzyme was the same as that for the enzyme present in the serum of patients with metastatic prostatic carcinoma (pH 5 with phenylphosphate as substrate) The carcinoma enzyme was much more effective in catalyzing hydrolysis of $\beta$ glycerophosphate

In a number of other patients with Niemann Pick disease the serum acid phosphatase level has been normal when phenylphosphate at pH 5 has been used as substrate [22] Thus the finding of a moderate elevation in the concentration of this enzyme appears to be much le s common than in Gaucher s disease The possible relationship of this phenomenon to the etiology of either of these conditions remains to be determined

is active in catalyzing the esterification of ceramide containing either short or long chain N acyl groups

An interesting feature of the in vitro system used by Sribney and Kennedy [65] is the requirement that the sphingosine of active ceramide must have the *trans* configuration of the double bond and the hydroxyl group on carbon 3 must have the *threo* relationship to the amino group on carbon 2 Most of the naturally occurring sphingosine is in the *erythro* form and the implications of this including a possible relationship to Niemann Pick disease are discussed further below

Studies on the synthesis of phospholipids mainly as determined by the incorporation of labeled phosphorus have been made in a variety of systems Some of these particularly dealing with the brain have been reviewed by Dawson [66] Very few studies have been made on the synthesis of sphingomyelin in vivo [67] There are no published experiments comparing synthetic rates in Niemann Pick disease with the normal

## CATABOLISM OF SPHINGOMYELIN

Very little is known about the dissimulative enzymes which may degrade sphingomyelin and the pathways which the products subsequently follow There are several points at which the molecule is especially subject to attack These include the liberation of choline by a cholinesterase followed by release of inorganic phosphate or removal of phosphoryl choline as a unit and the splitting of fatty acid from the ceramide portion Only the removal of phosphoryl choline by enzymatic means has been demonstrated

Over 20 years ago Rossi [68] reported the release of inorganic phosphate from sphingomyelin by an extract of spleen Similar studies using liver and brain extracts were reported by Thannhauser and Reichel [69] and Goebel and Seckfort [70] Rossi's results have been confirmed more recently by Fujino [71] who showed that splenic extracts were capable of splitting the phosphate-ceramide bond but did not attack the N acyl linkage This sphingomyelinase activity appears identical to that of certain phospholipase C enzymes which have been isolated from a number of sources

Phospholipase is the generic term adopted for enzymes acting on lipid phosphatides Much of the available information concerning them has been summarized by Zeller [72] Hanahan [73] and Hayaishi [74] These enzymes are commonly named phospholipase A B C or D according to their point of attack on lecithin taken as the model substrate [72] Phospholipases A and B which are esterases attacking the glycerol–fatty acid ester bonds of glycerophosphatides do not have any activity against the peptidic fatty acid linkage in sphingomyelin [72 [illegible]]

Phospholipase C isolated from *Clostridium welchii* [[illegible]] and *Clostridium hemolyticum* [76] catalyzes the hydrolysis of phosphorylcholine from both

generation (the fragmentation and loss of myelin which follows sectioning of a peripheral nerve) it has been suggested that one of the earliest changes might be the splitting of choline from sphingomyelin, since a rise in the content of ceramide phosphate has been observed [62]

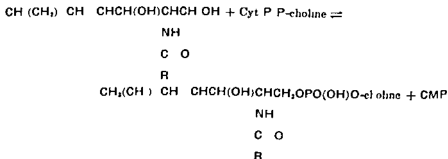
Sphingomyelin comprises roughly 5 to 20 per cent of the total phospholipids in the liver and other viscera It is also an important constituent of the stroma of blood cellular elements [63] The absolute and relative concentrations of sphingomyelin have been reported to be higher in (rabbit) leukocytes than erythrocytes [64] In plasma of man and most species sphingomyelin accounts for 15 to 20 per cent of the total phospholipid The same proportion holds for other extracellular fluid [27]

Aside from probably important roles in maintaining the structural integrity of the myelin sheath the lipoproteins and other cellular membranes other specific functions for sphingomyelin have not been suggested

## BIOCHEMISTRY

The sequential steps through which the synthesis of sphingomyelin normally proceeds in all tissues are not known Presumably the pathway may proceed from sphingosine to ceramide and finally to sphingomyelin It has been suggested [53] that at least in nervous tissue gangliosides might be precursors or more specifically ceramide donors for sphingomyelin synthesis This speculation and the synthesis of sphingosine and ceramide are discussed in more detail in Chap 17

Regardless of these uncertainties it has been demonstrated by Sribney and Kennedy [65] that the enzymatic synthesis of sphingomyelin from ceramide and phosphorylcholine does occur in a number of tissues The addition of 1 choline is accomplished in a manner analogous to the synthesis of lecithin (Fig 16-4) through the cytidine diphosphate choline pathway




The enzyme catalyzing this transformation PC ceramide transferase has been found so far in cell free particulate preparations of a number of tissues in several species including liver kidney spleen and brain The highest activity has been found in chicken liver The chicken liver en

is active in catalyzing the esterification of ceramide containing either short or long-chain N acyl groups

An interesting feature of the in vitro system used by Sribney and Kennedy [65] is the requirement that the sphingosine of active ceramide must have the *trans* configuration of the double bond and the hydroxyl group on carbon 3 must have the *threo* relationship to the amino group on carbon 2 Most of the naturally occurring sphingosine is in the *erythro* form and the implications of this including a possible relationship to Niemann Pick disease are discussed further below

Studies on the synthesis of phospholipids mainly as determined by the incorporation of labeled phosphorus have been made in a variety of systems Some of these particularly dealing with the brain have been reviewed by Dawson [66] Very few studies have been made on the synthesis of sphingomyelin in vivo [67] There are no published experiments comparing synthetic rates in Niemann Pick disease with the normal

## CATABOLISM OF SPHINGOMYELIN

Very little is known about the dissimulative enzymes which may degrade sphingomyelin and the pathways which the products subsequently follow There are several points at which the molecule is especially subject to attack These include the liberation of choline by a cholinesterase followed by release of inorganic phosphate or removal of phosphoryl choline as a unit and the splitting of fatty acid from the ceramide portion Only the removal of phosphoryl choline by enzymatic means has been demonstrated

Over 20 years ago Rossi [68] reported the release of inorganic phosphate from sphingomyelin by an extract of spleen Similar studies using liver and brain extracts were reported by Thannhauser and Reichel [69] and Goebel and Seckfort [70] Rossi s results have been confirmed more recently by Fujino [71] who showed that splenic extracts were capable of splitting the phosphate-ceramide bond but did not attack the N acyl linkage This sphingomyelinase activity appears identical to that of certain phospholipase C enzymes which have been isolated from a number of sources

Phospholipase is the generic term adopted for enzymes acting on lipid phosphatides Much of the available information concerning them has been summarized by Zeller [72] Hanahan [73] and Hayaishi [74] These enzymes are commonly named phospholipase A B C or D according to their point of attack on lecithin taken as the model substrate [72] Phospholipases A and B which are esterases attacking the glycerol-fatty acid ester bonds of glycerophosphatides do not have any activity against the peptidic fatty acid linkage in sphingomyelin [72 74]

Phospholipase C isolated from *Clostridium welchii* [75] and *Clostridium hemolyticum* [76] catalyzes the hydrolysis of phosphorylcholine from both

lecithin and sphingomyelin Phospholipase D which splits choline from lecithin has so far been isolated from plant sources only [77] and its activity against sphingomyelin has not been reported It should be noted that a number of authors, following the nomenclature proposed early by Contardi and Ercoli [78] reverse the terminology used above for phospholipase C and D activity

A comparison of phospholipase C activity (or "lecithinase D activity" in the terminology preferred by the authors [22]) in normal and Niemann Pick spleens has recently been made by Crocker and Farber [22] Extracts of spleen were incubated with a lecithin suspension and the release of acid soluble phosphorus determined There was no detectable difference between the activities of the extracts from the normal and abnormal tissues They also found that the sphingomyelin in the Niemann Pick tissues proved to be a readily utilizable substrate for *C welchii* phospholipase C Experiments similar to the latter had also been reported earlier by Freudenberg [79]

As has been pointed out by Thannhauser [27] and other authors the ceramide released from sphingomyelin is theoretically a pivotal compound and possibly available for utilization for synthesis of other sphingolipids This possible interconvertibility has been discussed in Chap 17 It is extremely difficult to devise experiments in vitro with sphingomyelin and related compounds which may duplicate the equilibriums and other conditions obtaining in the whole organism The substrates are relatively water insoluble and the activity of certain of the phospholipases has been shown to be influenced greatly by a number of factors including the presence of phospholipids other than the major substrate [80] The elucidation of the pathways with which Niemann Pick disease is intimately concerned is thus a problem of considerable magnitude

## DIAGNOSIS

Niemann Pick disease may be strongly suspected from the combination of hepatosplenomegaly and neurologic signs and symptoms A tentative diagnosis can be made from the additional finding of NP cells in bone marrow aspirates or tissue biopsies The diagnosis should be confirmed by the chemical determination of a predominant increase in sphingomyelin in the involved tissues

This point is emphasized particularly because of the interesting report of Baar and Hickmans [33] of a disease in two siblings which might have passed as Niemann Pick disease had analyses of tissues not been made The clinical features were compatible with the latter diagnosis although the NP like cells yielded a peculiar color with the Smith Dietrich reaction The abnormally elevated lipid in the organs proved to be an inosamine-phosphatide The plasma phospholipid partition was also abnormal It is

conceivable that this 'cephalin lipidosis' or another similar disorder has already been erroneou ly included among the ca es of "Niemann Pick disease which have been reported

It is also po sible of cour e that not all cases of sphingomyelin lipidosis arise from the same biochemical disorder In every case specimens of tissues should be carefully stored by refrigeration for future reference Such tissues are relatively rare and only by examining them with each appropriate new technique for determining lipid or enzyme content will the cause of this distressing syndrome be ascertained

A rather simple method for determining the sphingomyelin content of tissues u eful in laboratories equipped to do lipid phosphorus determina tions has been described [22] It is based on techniques developed by Schmidt and coworkers which are presented more extensively elsewhere [81] Lea Rhodes and Stoll first described the successful separation of pho pholipids on silicic acid columns [82] and recent papers by Hanahan et al [83] and Philips [84] are repre entative of further modifications of this technique made by many laboratories Marinetti et al [85] have described useful systems for quantitative paper chromatography of lipid pho phatides

## TREATMENT

There is no specific treatment at pre ent Splenectomy repeated blood transfusions p-aminosalicylic acid nitrogen mustard folic acid an tagonists lipotropic substances cortisone antibiotics liver extract and massive doses of vitamin A have been tried without remarkable effect on the progress of the disease [22] Anterior pituitary extract was reported to have reduced the size of the spleen and to have ameliorated some of the symptoms in one case [28] Radiation may be helpful in local control of infiltrative skin lesions [22]

## ETIOLOGIC POSSIBILITIES

The absence of inflammatory or other detectable signs of a di ease to which the accumulation of phingomyelin and other lipids might be secondary makes it extremely likely that Niemann Pick disease is a primary lipidosis Two patients with apparently primary carcinoma of the liver associated with wide pread occurrence of NP like cells [22 86] and increased phingomyelin content of other organs [22] have been reported In one [22] severe hyperlipidemia was also ob erved The c findings are not enlightening so far as the etiology of Niemann Pick di ease is concerned except to empha ize that similar tis ue changes may have a polyvalent basis

A defect in phingomyelin metaboli m is most highly u pect as the

lecithin and sphingomyelin Phospholipase D which splits choline from lecithin has so far been isolated from plant sources only [77] and its activity against sphingomyelin has not been reported It should be noted that a number of authors, following the nomenclature proposed early by Contardi and Ercoli [78] reverse the terminology used above for phospholipase C and D activity

A comparison of phospholipase C activity (or ' lecithinase D activity in the terminology preferred by the authors [22]) in normal and Niemann Pick spleens has recently been made by Crocker and Farber [22] Extracts of spleen were incubated with a lecithin suspension and the release of acid soluble phosphorus determined There was no detectable difference between the activities of the extracts from the normal and abnormal tissues They also found that the sphingomyelin in the Niemann Pick tissues proved to be a readily utilizable substrate for *C welchii* phospholipase C Experiments similar to the latter had also been reported earlier by Freudenberg [79]

As has been pointed out by Thannhauser [27] and other authors the ceramide released from sphingomyelin is theoretically a pivotal compound and possibly available for utilization for synthesis of other sphingolipids This possible interconvertibility has been discussed in Chap 17 It is extremely difficult to devise experiments in vitro with sphingomyelin and related compounds which may duplicate the equilibriums and other conditions obtaining in the whole organism The substrates are relatively water insoluble and the activity of certain of the phospholipases has been shown to be influenced greatly by a number of factors including the presence of phospholipids other than the major substrate [80] The elucidation of the pathways with which Niemann Pick disease is intimately concerned is thus a problem of considerable magnitude

## DIAGNOSIS

Niemann Pick disease may be strongly suspected from the combination of hepatosplenomegaly and neurologic signs and symptoms A tentative diagnosis can be made from the additional finding of NP cells in bone marrow aspirates or tissue biopsies The diagnosis should be confirmed by the chemical determination of a predominant increase in sphingomyelin in the involved tissues

This point is emphasized particularly because of the interesting report of Baar and Hickmans [33] of a disease in two siblings which might have passed as Niemann Pick disease had analyses of tissues not been made The clinical features were compatible with the latter diagnosis although the NP like cells yielded a peculiar color with the Smith Dietrich reaction The abnormally elevated lipid in the organs proved to be an inosaminephosphatide The plasma phospholipid partition was also abnormal It is

conceivable that this cephalin lipidosis' or another similar disorder has already been erroneously included among the cases of 'Niemann Pick disease which have been reported

It is also possible of course that not all cases of sphingomyelin lipidosis arise from the same biochemical disorder *In every case specimens of* tissues should be carefully stored by refrigeration for future reference Such tissues are relatively rare and only by examining them with each appropriate new technique for determining lipid or enzyme content will the cause of this distressing syndrome be ascertained

A rather simple method for determining the sphingomyelin content of tissues useful in laboratories equipped to do lipid phosphorus determinations has been described [22] It is based on techniques developed by Schmidt and coworkers which are presented more extensively elsewhere [81] Lea Rhodes and Stoll first described the successful separation of phospholipids on silicic acid columns [82] and recent papers by Hanahan et al [83] and Philips [84] are representative of further modifications of this technique made by many laboratories Marinetti et al [85] have described useful systems for quantitative paper chromatography of lipid phosphatides

## TREATMENT

There is no specific treatment at present Splenectomy repeated blood transfusions p aminosalicylic acid nitrogen mustard folic acid antagonists lipotropic substances cortisone antibiotics liver extract and massive doses of vitamin A have been tried without remarkable effect on the progress of the disease [22] Anterior pituitary extract was reported to have reduced the size of the spleen and to have ameliorated some of the symptoms in one case [28] Radiation may be helpful in local control of infiltrative skin lesions [22]

## ETIOLOGIC POSSIBILITIES

The absence of inflammatory or other detectable signs of a disease to which the accumulation of sphingomyelin and other lipids might be secondary makes it extremely likely that Niemann Pick disease is a primary lipidosis Two patients with apparently primary carcinoma of the liver associated with widespread occurrence of NP like cells [22 86] and increased sphingomyelin content of other organs [22] have been reported In one [22] severe hyperlipidemia was also observed These findings are not enlightening so far as the etiology of Niemann Pick disease is concerned except to emphasize that similar tissue change may have a polyvalent basis

A defect in sphingomyelin metabolism is most highly suspect as the

cause of the primary lipidosis In assuming that this is the site of the metabolic error, it is necessary also to explain the excessive concentrations of cholesterol and other lipids other than sphingomyelin in most of the tissues examined It is not possible to do this at present There are however convenient examples among the various hyperlipidemias (Chap 16) suggesting that a single lipid is rarely if ever uniquely elevated in the serum This is presumably because of the requirements imposed by lipoprotein structure, probably similar requirements obtain in tissues as well This analogy may be carried further of course by recalling suggestions (Chap 16) that excessive elaboration of a protein with special affinity for lipid might be responsible for certain types of hyperlipidemia This concept has been advanced by Uzman [87] as being related to the cause of another sphingolipidosis Gaucher s disease Search for evidence of abnormal protein binding of lipid in Niemann Pick tissues has not been successful [22]

It does not seem likely that an increased net transport of sphingomyelin through the extracellular fluid from a single site to all other organs is important Thannhauser [27] has stressed that normal plasma concentrations of this lipid minimize this possibility Regardless of whether or not there is increased sphingomyelin transport it must be secondary to some other abnormality in the production or degradation of lipid Several experiments have been conducted in which sphingomyelin emulsions have been injected into animals [88–90] Cells similar to the NP cell were said to have developed in several tissues but on at least one occasion they were not reproduced [91] and the experiments do not shed much light on pathogenesis It is surprising indeed that there is no increase in sphingomyelin in extracellular fluid The reticuloendothelial cells seem to retain to the end a remarkable affinity for the excess lipid

In consideration of a defect in sphingomyelin metabolism three or four major possibilities exist For and against each some intuitive argument or fragmentary experimental evidence may be raised The first is that the synthesis of a normal sphingomyelin molecule proceeds unchecked and at a reckless pace which far outstrips dissimulation of the molecule A possible analogy is gout (Chap 21) in which both abnormal diversion of substrate to the product uric acid and a faulty control mechanism are suspected

A second possibility is the synthesis of a structurally abnormal sphingomyelin molecule resistant to the action of a normal complement of degradative enzymes One possibility of this nature has been raised by Sribney and Kennedy [65] who found that only ceramide containing sphingosine in the *threo* configuration could serve as substrate for their PC ceramide transferase reaction All naturally occurring spingosine appears to be in the *erythro* form [92] and Sribney and Kennedy postulated that isomerization might be a final step in normal sphingomyelin

synthesis They further called attention to the possibility that such a step could be missing in Niemann Pick tissues and that the resulting *threo* sphingomyelin might be resistant to further catabolism Neither such a normal isomerase reaction nor the stereoisomerism of the sphingomyelin in Niemann Pick tissues has yet been established As noted earlier the fatty acid composition of sphingomyelin must also be determined with greater accuracy One argument against the possibility of an important alteration in sphingomyelin is the apparent availability of this molecule in Niemann Pick tissues as a substrate for phospholipase C hydrolysis [22]

As mentioned above and in Chaps 17 and 19 in ganglioside and cerebroside lipidoses an abnormal macromolecular structure or protein binding of the offending lipid has been suggested as the underlying abnormality Polydiaminophosphatides were described many years ago in lipid isolates from tissue [68 93] but it is probable that these were artifacts [94] and in the intervening years no further evidence that sphingomyelin might exist in tissues in any type of polymer has been presented

The final possibility seems the most likely that there exists in Niemann Pick disease some inheritable alteration in or absence of an important dissimulative enzyme Phospholipase C activity is present in these tissues [22] as it is in normal tissue but beyond this nothing is known of the routes or products of sphingomyelin catabolism Until the normal pathways are established it is impossible to speculate knowingly on possible single enzyme defects that might account for Niemann Pick disease

The available evidence indicates that whatever the basic defect it expresses itself widely perhaps involving most tissue cells Presumably the dramatic and early [32] involvement of the reticuloendothelial system indicates that the responsible defect is present in these cells and that they are not merely exercising their well known capacity for lipid phagocytosis Nothing definite is known of the relative capacity of reticuloendothelial cells to make and degrade sphingomyelin Of interest are the post mortem findings in a stillborn infant with Niemann Pick disease examined by Burne [95] The most advanced changes were present in the spleen thymus lymph nodes and adrenal cortex No foam cells were present in the liver but the parenchymal cells were distended with glycogen and Kupffer cells were swollen The organ was not analyzed chemically In the kidney no deposition of lipid was detected histologically but it contained abnormally large amounts of sphingomyelin Autonomic ganglion cells throughout the body were undergoing typical destructive changes and in the brain the ganglion cells of the pons were abnormal Those sections of the cerebellum and cerebral cortex which were examined appeared normal

It appears then that lipid begins to accumulate first not only in tissues

rich in reticulum cells but also in the more "primitive" ganglion cells Such early predilection may be an expression of an advanced functional capacity of these cells In the brain, sphingomyelin begins to accumulate after birth [*62, 96*] and major changes in the cerebral cortex would be expected to be somewhat delayed

## RELATIONSHIP TO TAY SACHS DISEASE

The changes in the brain in Niemann Pick disease afford an especially interesting comparison with those in infantile amaurotic family idiocy (Tay Sachs disease) (Chap 17) This is especially true with respect to the relationship suggested between gangliosides elevated in Tay Sachs disease and sphingomyelin It has been considered that in the brain at least gangliosides might be the precursor of sphingomyelin [*53*] the latter being elaborated mainly by the axon or glial cell responsible for the laying down of myelin Yet in Niemann Pick disease it is remarkable that the ganglion cells themselves seem to be distended or "ballooned until they die resulting in demyelination or failure to myelinate in a manner identical to that in Tay-Sachs disease The only difference in the brain would seem to be in the lipid accumulated The establishment of the pathways relating sphingomyelin and gangliosides is awaited with interest in this regard The occurrence of the two lipidoses in the same pedigree has been reported [*97*] but the diagnoses were not substantiated by chemical analyses Until such evidence is obtained there is no firm basis for assuming that the two diseases are very similar biochemically e g involving the same enzyme system with some other determinant directing the nature of the abnormal lipid which accumulates

## GENETICS

Although Niemann Pick disease is rare it has been observed too frequently among siblings to be due to chance In a total of 91 cases [*21 22*] at least 12 have involved more than one patient in a sibship Three siblings have been affected in at least two families [*22 48*] There are in addition a number of families in which one or more siblings of the proband have died following an illness suggestive of but not proved to be Niemann Pick disease The sex distribution is about equal

More cases have occurred in Jews than in any other ethnic group—35 of 73 cases in which the race is known in the two largest series [*21 22*] Most of the remaining cases have occurred in other Caucasians There has been one case in a child of Syrian parentage [*22*] and one definite another probable case from the mating of an American Indian Negro father and a Portuguese mother [*22*] Cases have also been reported from Turkey [*98*] and Japan [*99*] Of special interest are four cases of Crocker and Farber [*22*] all of French Canadian Catholic ancestry who had a

similar and unusually protracted course There was no traceable inter breeding but the same family name occurred in several of the pedigrees

Of 79 matings producing at least one child with Niemann Pick disease [21 22 28 100] six are known to have been consanguineous It is difficult to be certain of the rate of occurrence in involved families particularly as has been pointed out [22] since parents may tend to limit the size of their families after the appearance of an involved offspring Crocker and Farber [22] found 17 definite cases in a total of 45 contemporaries among their 13 families with more than one child No doubt because of its semi lethal character the disease has not been reported in more than one generation in an affected pedigree Thus the available evidence indicates that Niemann Pick disease is probably inherited as an autosomal recessive trait [101] but this has not been firmly established

Some attention has been given to the problem of detecting heterozygous carriers although in many cases no family study at all has been reported Two pedigrees have been examined by Pfandler [28] The first is that from the Brevine valley in Switzerland from which came the first two cases reported in adults These two brothers whose disease was proved at autopsy had 12 siblings Two of these al o had hepatosplenomegaly but were over 50 years of age at the time of examination Pfandler con idered these two siblings as having latent macrosymptoms of the disease Several other members of this pedigree were found to have an increase in serum total fatty acids The cholesterol values were normal and the pho pholipids not determined The same type of hyperlipidemia was described in the siblings and a parent of a Danish family in which two definite cases of Niemann Pick disease appeared These changes in the blood lipids which Pfandler calls microsymptoms are interesting but require further evaluation

In two separate cases both sets of parents were found to have normal bone marrows and serum lipids by Crocker and Farber In one case [22] the father and two paternal granduncles had splenomegaly In one of the latter bone marrow and splenic aspirations revealed abnormal cells differing from NP cell but comparable to others which have been previ ously described [102] A search for vacuolization of agranulocytes in the peripheral blood in parents and siblings of several cases has not been rewarding [22]

Until a clear-cut trait can be defined it will be difficult not only to determine the preci e mode of inheritance of Niemann Pick di ease but also to solve the related problem of whether more than one biochemical di order may be currently considered as a single disease

## SUMMARY

1 Niemann Pick di ea e is an inheritable condition in which lipid accumulates in reticuloendothelial and other cell types throughout the

body Although every organ may be involved the changes occurring in the nerve ganglion cells are especially destructive leading to cell death and demyelination

2 The clinical features include hepatosplenomegaly retarded physical and mental growth and severe neurologic disturbances A cherry red spot in the macula may be present Symptoms usually develop by 6 months of age and the disease is usually fatal by the third year Some cases beginning in infancy may survive much longer Sporadic cases have been detected in adults

3 The accumulated lipid in the tissues is mainly sphingomyelin Other phospholipids and cholesterol may also be present in abnormally high concentration

4 The disease is probably transmitted as an autosomal recessive trait It is most common in children of Jewish parentage

5 The underlying biochemical disorder most likely a defect in sphingomyelin metabolism is unknown

## BIBLIOGRAPHY


1 Niemann A Ein unbekanntes Krankheitsbild Jahrb Kinderh 79 1 1914
2 Knox J H M Jr Wahl H R and Schmeisser H C Gaucher s disease a report of two cases in infants Bull Johns Hopkins Hosp 27 1 1916
3 Siegmund H Lipoidzellenhyperplasie der Milz und Splenomegalie Gaucher Verhandl deutsch path Gesellsch 18 59 1921
4 Mandlebaum F S and Downey H The cases of Gaucher s disease reported by Drs Knox Wahl and Schmeisser Bull Johns Hopkins Hosp 27 109 1916
5 Pick L Über die lipoidzellige Splenohepatomegalie Typus Niemann Pick als Stoffwechselerkrankung Med Klin 23 1483 1927
6 Pick L II Niemann Pick s disease and other forms of so-called xanthomatoses Am J M Sc 185 601 1933
7 Wahl H R and Richardson M L A study of the lipin content of a case of Gaucher s disease in an infant Arch Int Med 17 238 1916
8 Bloom W and Kern R Spleens from Gaucher s disease and lipoid histiocytosis the chemical analysis Arch Int Med 39 456 1927
9 Brahn B and Pick L Zur chemischen Organanalyse bei der lipoidzelligen Splenohepatomegalie Typus Niemann Pick Klin Wchnschr 6 2367 1927
10 Corcan I Oberling C and Dienst G La maladie de Niemann Pick Rev franç pédiat 3 789 1927
11 McFate R I The chemical analysis of liver and spleen from a case of lipoid histocytosis (Niemann Pick s disease) Arch Path & Lab Med 6 1004 1928
12 Epstein E and Lorenz K Die Phosphatidzellverfettung in Gehirn Leber und Milz bei Niemann Pick chen Krankheit Ztschr physiol Chem 211 217 1932
13 Sobotka H Glick D Reiner M and Tuchman L R The lipoids of spleen and liver in various types of lipoidosis Biochem J 27 2031 1933
14 Klenk E Über die Natur der Phosphatide der Milz bei der Niemann Pick chen Krankheit Ztschr physiol Chem 229 151 1934
15 Klenk E Über die Natur der Phosphatide und anderer Lipoide des Gehirns und der Leber bei der Niemann Pickschen Krankheit Ztschr physiol Chem 235 24 1935
16 Tropp C and Eckhardt B Sphingomyelin bei Niemann Pickschen Krankheit Ztschr physiol Chem 243 38 1936
17 Tropp C and Eckhardt B Gehirn Sphingomyelin bei Niemann Pickschen Krankheit Ztschr physiol Chem 245 163 1936

18 Teunis en P H and Den Ouden A Nachtrag zu der Mitteilung Beitrag zur Kenntnis der Ch mie der Lipoi losis phosphatidica Ztschr physiol Chem 252 71 1938
19 Thannhau er S J B notti J and Rein tein H as cited by Thannhauser S J *Lipid s s Diseas s of the Intracell ılar Lipi l Metabol sm* Grune & Stratton New York 1958
20 Chargaff E A study of the spleen in a case of Niemann Pick disea e J Biol Chem 130 503 1939
21 Videbaek A Niemann Pick s disease Acute and chronic type? Acta paediat 37 95 1949
22 Crocker A C and Farber S Niemann Pick disease a review of 18 patients Medicine 37 1 1958
23 Dusendschon A *Deux cas familiaux de maladie de Niema n Pick che adulte* These Faculté de médicine Geneva 1946
24 Pfandler U La maladie de Niemann Pick dans le cadre des lipoidoses Schweiz med Wchnschr 76 1128 1946
25 Terry R. Sperry W M and Brodoff B Adult lipidosis resembling Niemann Pick s disease Am J Path 30 263 1954
26 Miller F L. Personal communication
27 Thannhauser S J *Lipidoses D seases of the Intracellular Lipid Metabolism* Grune & Stratton New York 1958
28 Pfandler U Nouvelles conceptions sur l hérédité et la pathogénie de la maladie Niemann Pick Helvet med acta 20 216 1953
29 Baumann T Klenk E and Scheidegger S Niemann Pickschen Krankheit eine klinische chemische und histopathologische Studie Ergebn allg Path u path Anat 30 183 1936
30 Canmann M F Niemann Pick disea e (acute idiopathic xanthomatosis phosphatide lipidosis l poid cell splenomegaly–Niemann type) J Pediat 24 335 1944
31 Maurer L E Niemann Pick s disease a report of four cases Rocky Mt M J 38 460 1941
32 Bloom W The hi togenesis of essential lipoid histiocytosis (Niemann Pick s disease) Arch Path 6 8 7 19 8
33 Baar H S and Hickmans E M Cephalin lipidosis a new d sorder of lipid metabolism Acta med scand nav 155 49 1956
34 Diezel P B Histochemische Untersuchung n an primären Lipoidosen am aurotı che Idiotie Gargoylismus Niemann Pickschen Krankheit Gauchersche Krankheit mit besonderer Berucksichtigung des Zentralnervensystems Arch path Anat 326 89 1954
35 Landing B H and Freiman D G Histochemical studies on the cerebral lipido es and other cellular metabol c disorders Am J Path 33 1 1957
36 Diez l P B Hi tochemical study of primary lipidoses in *Cerebral Lip doses* edited by L. van Bogaert J N Cumings and A Lowenthal p 11 Blackwell Oxford 1957
37 Hassin G B Niemann Pick s di ea e Pathologic stud es of a case Arch Neurol & Psychiat 24 61 1930
38 Kutti R E Niemann Pick s disea e and its relationsh p to the lipoidoses California Med 72 60 1950
39 Pick L. and Biel chowsky M Über lipoidzellige Splenomegalie (Typus Niemann Pick) und amaurotische Idiotie Klin Wchr chr 6 1631 1927
40 Drus J G Pathologic changes in the e r in Niemann Pick s d sease Arch Otolaryng 16 592 193
41 Wascowitz B Niemann Pi k s d se se (essential lipo d histiocytosis) Am J Dis Ch l l 42 3 6 1931
42 Rothstein J I and Welt S Infant le amaurotic family idiocy Am J Dis Child 62 801 1941
43 Goll tein I and We ler D N emann P ck s disea e with cherry red spots in the macula ocular pathology Arch Ophth (Chicago) 5 704 1931
44 Rintelen F Die H topath logie der Augenh ntergrunds veranderungen bei

body Although every organ may be involved the changes occurring in the nerve ganglion cells are especially destructive leading to cell death and demyelination

2 The clinical features include hepatosplenomegaly, retarded physical and mental growth and severe neurologic disturbances A cherry red spot in the macula may be present Symptoms usually develop by 6 months of age and the disease is usually fatal by the third year Some cases beginning in infancy may survive much longer Sporadic cases have been detected in adults

3 The accumulated lipid in the tissues is mainly sphingomyelin Other phospholipids and cholesterol may also be present in abnormally high concentration

4 The disease is probably transmitted as an autosomal recessive trait It is most common in children of Jewish parentage

5 The underlying biochemical disorder most likely a defect in sphingomyelin metabolism is unknown

## BIBLIOGRAPHY


1 Niemann A. Ein unbekanntes Krankheitbild Jahrb Kinderh 79 1 1914
2 Knox J H M Jr Wahl H R and Schmeisser H C. Gaucher's disease a report of two cases in infants Bull Johns Hopkins Hosp 27 1 1916
3 Siegmund H Lipoidzellenhyperplasie der Milz und Splenomegalie Gaucher Verhandl deutsch. path Gesellsch 18 59 1921
4 Mandlebaum F S and Downey H The cases of Gaucher's disease reported by Drs Knox Wahl and Schmeisser Bull Johns Hopkins Hosp 27 109 1916
5 Pick L. Über die lipoidzellige Splenohepatomegalie Typus Niemann Pick als Stoffwechselerkrankung Med. Klin 23 1483 1927
6 Pick, L. II Niemann Pick's disease and other forms of so-called xanthomatoses Am J M Sc 185 601 1933
7 Wahl H R and Richardson M L. A study of the lipin content of a case of Gaucher's disease in an infant Arch Int Med 17 238 1916
8 Bloom W and Kern R Spleens from Gaucher's disease and lipoid histiocytosis the chemical analysis Arch Int Med 39 456 1927
9 Brahn B and Pick L. Zur chemischen Organanalyse bei der lipoidzelligen Splenohepatomegalie Typus Niemann Pick Klin Wchnschr 6 236 1927
10 Corcan P Oberling C and Dienst G La maladie de Niemann Pick Rev franc pediat 3 789 1927
11 McFate R. P The chemical analysis of liver and spleen from a case of lipoid histiocytosis (Niemann Pick's disease) Arch Path & Lab Med 6 1064 1928
12 Epstein E and Lorenz K Die Phosphatidzellverfettung in Gehirn Leber und Milz bei Niemann Pickschen Krankheit Ztschr physiol Chem 211 21 1932
13 Sobotka, H Glick, D Reiner M and Tuchman L. R. The lipoids of spleen and liver in various types of lipoidoses Biochem J 27 2031 1933
14. Klenk E Über die Natur der Phosphatide der Milz bei der Niemann Pickschen Krankheit Ztschr physiol Chem 229 151 1934
15 Klenk E Über die Natur der Phosphatide und anderer Lipoide des Gehirns und der Leber bei der Niemann Pickschen Krankheit Ztschr physiol Chem 235 24 1935
16 Tropp C and Eckhardt B Sphingomyelin bei Niemann Pickschen Krankheit Ztschr physiol Chem 243 38 1936
17 Tropp C and Eckhardt B Gehirn Sphingomyelin bei Niemann Pickschen Krankheit Ztschr physiol Chem 245 163 1936

18 Teuni en P H and Den Ouden A Nachtrag zu der Mitteilung Beitrag zur Kenntnis der Chemie der Lipoido is phosphatidica Ztschr physiol Chem 252 2,1 1938
19 Thannhauser S J Benotti J and Reinstein H as cited by Thannhau er S J *Lipidoses Diseases of the Intracellular Lipid Metabolism* Grune & Stratton New York 1958
20 Chargaff E A study of the spleen in a case of Niemann Pick disease J Biol Chem 130 503 1939
21 Videbaek A Niemann Pick s disease Acute and chronic type? Acta paediat 37 95 1949
22 Crocker A C and Farber S Niemann Pick disease a review of 18 patients Medicine 37 1 1958
23 Dusendschon A *Deux cas familiaux de maladie de Niemann Pick che adulte* These Faculté de médicine Geneva 1946
24 Pfandler U La maladie de Niemann Pick dans le cadre des lipoidoses Schweiz med Wchnschr 76 1128 1946
25 Terry R Sperry W M and Brodoff B Adult lipidosis resembling Niemann Pick s disease Am J Path 30 263 1954
26 Miller F L Personal communication
27 Thannhauser S J *Lipidoses Diseases of the Int acellular Lipid Metabolism* Grune & Stratton New York 1958
28 Pfandler U Nouvelles conceptions sur l hérédité et la pathogénie de la maladie *Niemann Pick Helvet med acta 20 216 1953*
29 Baumann T Klenk E and Scheidegger S Niemann Pi kschen Krankheit eine klinische chemische und histopathologi che Studie Ergebn allg Path u path Anat 30 183 1936
30 Canmann M F Niemann Pick disease (acute idiopathic xanthomatosis phos phatide lipidosis lipoid cell splenomegaly–Niemann type) J Pediat 24 335 1944
31 Maurer L E Niemann Pick s disease a report of four cases Rocky Mt M J 38 400 1941
32 Bloom W The histogenesis of essential lipoid histiocytosis (Niemann Pick s d ease) Arch Path 6 827 1928
33 Baar H S and Hickmans E M Cephalin lipidosis a new disorder of lipid metabolism Acta med scandinav 155 49 1956
34 Diezel P B Histochemische Untersuchungen an primaren Lipoidosen am aurotische Idiotie Gargoylismus Niemann Pickschen Krankheit Gauchersche Krankheit mit besonderer Berücksichtigung des Zentralnervensystems Arch path Anat 326 89 1954
35 Landing B H and Freiman D G Histochemical stud es on the cerebral lipidoses and other cellular metabolic disorders Am J Path 33 1 1957
36 Diezel P B Histochemical study of primary lipidoses in *Cerebral Lipidoses* ed ted by L van Bogaert J N Cumings and A Lowenthal p 11 Blackwell Oxford 1957
37 Hassin G B Niemann Pick s disease Pathologic studies of a case Arch Neurol & Psychiat 24 61 1930
38 Knutti R E Niemann Pi k s di ea e and its relationship to the l po doses California Med 72 60 1950
39 Pick L and Bielschowsky M Über lipoidzellige Splenomegalie (Typus Nie mann Pick) und amaurotische Idiotie Klin Wchnschr 6 1631 192
40 D uss J G Pathologic changes in the ear in Niemann Pick s d ease Arch Otolaryng 15 59 193
41 Wa cowitz B N emann Pick s d ease ( ssential lipoid hi tiocytos ) Am J Dis Child 42 356 1931
42 Rothstein J L and Welt S Infantile amaurotic family idiocy Am J Dis Child 62 801 1941
43 Gold tein I and Wexler D Niemann Pick s disease with cherry red spots in the macula ocular pathology Arch Ophth (Chicago) 5 704 1931
44 Runtelen F De Histopathologie der Augenhintergrunds veranderungen bei

Niemann Pickschen Lipoidose Zugleich ein Beitrag zur Frage der Beziehungen zwischen Tay Sachscher Idiotie und Niemann Pickschen Lipoidose Arch Augenh 109 332 1935
45 Greenfield J G The retina in cerebrospinal lipidoses Proc Roy Soc Med 44, 686 1951
46 Abrikossoff A Demonstration eines eigenartigen Falles der Niemann Pickschen Krankheit Centralbl allg Path u path Anat 43 7 1928
47 Knox R A and Ramsey G W Niemann Pick's disease (essential lipid histiocytosis) Ann Int Med 6 218 1932
48 Merkamer D and Kramer B Niemann Pick's disease report of three cases in one family J Pediat 14 51 1939
49 Bloom W Splenomegaly (type Gaucher) and lipoid histiocytosis (type Niemann) Am J Path 1 595 1925
50 Klenk E Beiträge zur Chemie der Lipoidosen Niemann Pickschen Krankheit und amaurotische Idiotie Ztschr physiol Chem 262 128 1939
51 Klenk E Beiträge zur Chemie der Lipoidosen Ztschr physiol Chem 267 128 1940
52 Klenk E Über die Verteilung der Neuraminsäure im Gehirn bei der familiären amaurotischen Idiotie und bei der Niemann Pickschen Krankheit Ztschr physiol Chem 282 84 1947
53 Klenk E The pathological chemistry of the developing brain in *Biochemistry of the Developing Nervous System* edited by H Waelsch p 397 Academic Press Inc New York 1955
54 Cumings J N The diagnostic value of lipid estimations in the cerebral lipidoses in *Cerebral Lipidoses* edited by L van Bogaert J N Cumings and A Lowenthal p 112 Blackwell Oxford 1957
55 Bartsch G The nature of the lipids in the brain in Niemann Pick's disease in *Cerebral Lipidoses* edited by L van Bogaert J N Cumings and A Lowenthal p 153 Blackwell Oxford 1957
56 Hastrup B and Videbaek A Acid phosphatase in Niemann Pick's disease and a therapeutic experiment with cortisone Acta med scandinav 149 287 1954
57 Fujino Y Studies on the conjugated lipids III On the configuration of sphingomyelin J Biochem (Japan) 39 45 1952
58 Rouser G Berry J F Marinetti G V and Stotz E Studies on the structure of sphingomyelin I Oxidation of products of partial hydrolysis J Am Chem Soc 75 310 1953
59 Marinetti G Berry J F Rouser G and Stotz E Studies on the structure of sphingomyelin II Performic and periodic acid oxidation studies J Am Chem Soc 75 313 1953
60 Thannhauser S J and Boncoddo N The chemical nature of the fatty acids of brain and spleen sphingomyelin The occurrence of saturated and unsaturated sphingosines in the sphingomyelin molecule J Biol Chem 172 141 1948
61 Johnson A C McNabb A R and Rossiter R J Lipids of peripheral nerves Biochem J 43 578 1948
62 Brante B Studies on lipids in the nervous system with special reference to quantitative determination and topical distribution Acta physiol scandinav 18 Suppl 63 1 1949
63 *Yamakawa T and Suzuki S The chemistry of the lipids of post-hemolytic* residue or stroma of erythrocytes I Concerning the ether insoluble lipids of lyophilized horse blood stroma J Biochem (Japan) 38 199 1951
64 Burt N S and Rossiter R J Lipids of rabbit blood cells Data for red cells and polymorphonuclear *leucocytes Biochem J 46 569 1950*
65 Sribney M and Kennedy E P The enzymatic synthesis of sphingomyelin J Biol Chem 233 1315 1958
66 Dawson R M C The synthesis of neurophospholipides during growth in *Biochemistry of the Developing Nervous System* edited by H Waelsch p 268 Academic Press Inc New York 1955
67 Christensen S Jensen S E and Lundback K The incorporation of $P^{32}$ in phospholipid fractions in man Scandinav J Clin Lab Invest 7 212 1955

68 Rossi A Untersuchungen uber die Lipoide der Saugetiere IX Über die enzymatische Spaltbarkeit des Leber Polydiaminopho phatids Ztschr physiol Chem 231 115 1935
69 Thannhauser S J and Reichel M Studies on animal lipids XVI The occurrence of sphingomyelin as a mixture of sphingomyelin fatty acid ester and free sphingomyelin demonstrated by enzymatic hydrolysis and mild saponification J Biol Chem 135 1 1940
70 Goebel A and Seckfort H Über Spaltung von Sphingomyelinen durch Organe Biochem Ztschr 319 203 1948
71 Fujino Y Studies on the conjugated lipids IV On the enzymatic hydrolysis of sphingomyeline J Biochem (Japan) 39 55 1952
72 Zeller E A Enzymes as essential components of bacterial and animal toxins in *The Enzymes* edited by J B Sumner and K Myrback vol I part 2 p 986 Academic Press Inc New York 1951
73 Hanahan D J The lecithinases in *Progress in Chemistry of Fats and Related Compounds* vol 4 Pergamon Press 1957
74 Hayaishi O Phospholipases in *Methods in Enzymology* edited by Colowick S P and Kaplan N O vol 1 p 660 Academic Press Inc New York 1955
75 MacFarlane M G The biochemistry of bacterial toxins II The enzymic specificity of *Clostridium welchii* lecithinase Biochem J 42 587 1948
76 MacFarlane M G The biochemistry of bacterial toxins IV The lecithinase activity of *Clostridium haemolyticum* toxin Biochem J 47 267 1950
77 Hanahan D J and Chaikoff I L On the nature of the phosphorus-containing lipids of cabbage leaves and their relation to a phospholipide-splitting enzyme contained in these leaves J Biol Chem 172 191 1948
78 Contardi A and Ercoli A Über die enzymatische Spaltung der Lecithine und Lysocithine Biochem Ztschr 261 275 1933
79 Freudenberg F Klinische Beobachtungen und Untersuchen an einem Zwilling paar mit Niemann Pickschen Krankheit Ztschr Kinderh 59 313 1937
80 Dawson R M C Studies on the hydrolysis of lecithin by a *Penicillium notatum* phospholipase B preparation Biochem J 70 559 1958
81 Schmidt G Ottenstein B Spencer W A Keck K Blietz R Papas J Levin M L and Thannhauser S J The partition of tissue phospholipides by phosphorus analysis A M A J Dis Child 97 691 1959
82 Lea C H Rhodes D N and Stoll R D Phospholipids 3 On the chromatographic separation of glycerophospholipids Biochem J 60 353 1955
83 Hanahan D J Dittmer J C and Warashina E A column chromatographic separation of classes of phospholipides J Biol Chem 228 685 1957
84 Phillips G B The isolation and quantitation of the principle phospholipid components of human serum using chromatography on silicic acid Biochim et biophys acta 29 594 1958
85 Marinetti G V Erbland J and Kochen J Quantitative chromatography of phosphatides Fed Proc 16 837 1957
86 Wood H Generalized essential xanthomatosis (type Niemann Pick) associated with primary carcinoma of the liver in an infant Arch Path 26 873 1938
87 Uzman L L The lipoprotein of Gaucher s disease A M A Arch Path 61 3 1959
88 Baumer H and Gruber C Versuche zur experimentellen Erzeugung der Niemann Pick chen Krankheit Jahrb Kinderh 146 125 1936
89 Ferraro A and Jervis C A Studies in experimental lipoidosis I Phosphatides Arch Path 30 731 1940
90 Goebel A Das Verhalten von Sphingomyelinen im Tierkorper Biochem Ztschr 319 196 1948
91 Lettrer E Verhandl d ut ch path Gesell ch 30 183 1937 and 31 1938 cited by Goebel (ref 90)
92 Carter H E Sphingolipides in *Chemistry of Lipids as Related to Atherosclerosis* edited by I H Page p 82 Charles C Thomas Springfield Ill 1958
93 Frankel E Bielschowsky F and Thannhauser S J Untersuchungen über die

Lipoide der Säugetierleber III Über ein Polydiaminophosphatid der Schweineleber Ztschr physiol Chem 218 1 1933
94 Klenk E Über die Sphingomyeline des Herzmuskels Ztschr physiol Chem 221 67 1933
95 Burne J C Niemann Pick disease in a foetus J Path & Bact 66 473 1953
96 Folch J Composition of the brain in relation to maturation in *Biochemistry of the Developing Nervous System* edited by H Waelsch p 121 Academic Press Inc New York 1955
97 Van Bogaert L and Klein D Observations sur l hérédité des idioties amaurotiques et de la spléno hépatomégalie lipidienne (11 familles) J Genet Hum 4 23 1955
98 Facatselli N Ann paediat 162 218 1944 cited by Videbaek (ref 21)
99 Tokugawa H Tr Jap Path Soc 27 114 1937 cited by Videbaek (ref 21)
100 Videbaek A. Another case of Niemann Pick s disease observed in Denmark Acta paediat 41 355 1952
101 Herndon C N Genetics of the lipidoses Res Publ A Nerv & Ment Dis 33 239 1954
102 Sawitsky A Hyman G A and Hyman J B An unidentified reticulo endothelial cell in bone marrow and spleen Blood 9 977 1954

# Chapter 19

# Gaucher's Disease

---

*Donald S. Fredrickson and Alan F. Hofmann*

Gaucher's disease (cerebroside lipidosis, cerebrosidosis) is a rare familial disease in which characteristic cells loaded with cerebrosides are found in reticuloendothelial tissues. It may become clinically apparent at any age. In the adult it progresses slowly and the manifestations are directly related to enlargement and destruction of tissues filled with masses of Gaucher cells. When it appears in infancy, the process is more severe and is usually accompanied by crippling lesions in the nervous system which tend to hasten the course to an early death.

This condition was first described by Phillipe Gaucher in 1882 [1], and for over a half century the clinical course, relentless and monotonous in its reproducibility, has been well known. During this time significant advances have been made in disclosing the chemical abnormalities, but the basic metabolic lesion in Gaucher's disease is still unknown. This chapter will be devoted more to a review of the relevant biochemical area than to the fine detail of the clinical manifestations. These latter have been carefully reviewed by a number of authors [2–9], the comprehensive review of Thannhauser being the most recent [9].

## HISTORICAL ASPECTS

In his description of the first case, Gaucher called attention to the chronic progressive course, the splenomegaly, hepatomegaly, and skin pigmentation, and considered that the peculiar cells in the spleen, which were later to bear his name, were evidence of a primary neoplasm of that organ. He later revised this opinion [10], suggesting that the major process was connective tissue proliferation associated with enlargement of parenchymal cells. As more cases were discovered, the concepts of etiology gradually evolved through a theory of toxic origin [11], of diffuse systemic disease of the lymphatic hemopoietic system [10], to the deposi-

tion of a foreign substance in the reticulum cells [12] either carried there by the blood [13] or elaborated *in situ* [9]

Following Marchand's suggestion that the abnormal cells contained a foreign substance [12] a number of investigations indicated that this material was 'lipoid' in nature These early studies are thoroughly reviewed by Epstein [14] who working with Lorenz [14 15] found the Gaucher spleen to contain large amounts of alcohol soluble acetone-insoluble material Spurred by their findings Lieb [16–18] in 1924 isolated from this tissue large amounts of the cerebroside kerasin which had been described 50 years earlier in brain by Thudichum [19 20] Many subsequent analyses have brought confirmation that the typical lesion in Gaucher's disease contains excessive amounts of cerebroside In most cases however the cerebroside accumulating in the viscera is not the normal galactocerebroside but contains glucose This was first discovered by Aghion in 1934 [21] and confirmed by more certain analytical techniques by Halliday et al [22] in 1940 The finding of increased acid phosphatase activity in plasma and Gaucher tissues [23, 23a] is the most recent advance of possible major significance

The occurrence of Gaucher's disease in siblings was noted in very early case reports [11 24 25] and the few hundred case reports since published clearly indicate that the disease has some hereditary basis

## CLINICOPATHOLOGIC CORRELATIONS

The diagnosis of Gaucher's disease has been made at ages as disparate as 1 week [2] and 79 years [26] About one third of the reported cases have been detected in infancy or childhood The chemical abnormalities found in the tissues of so-called infantile childhood and adult forms have been indistinguishable It is assumed that the disease represents a continuum with significant differences in the nature of the manifestations depending upon the time of appearance or rate of development

In infancy the process leading to cerebroside accumulation is most widespread In addition to the usual enlargement of the spleen and liver marked lymph node involvement is common and Gaucher cells may appear in lung thymus adrenal cortex and other tissues [7] Most importantly there also occur destructive lesions in ganglion cells in the brain and the clinical course may be dominated by signs of gross central nervous system dysfunction These include poor feeding patterns laryngospasm spasticity and a variety of other abnormal signs [2] In addition there are mental retardation eventually total idiocy and cachexia and finally death often from intercurrent infections

In most children who first develop signs of the disease after the age of 6 months there are few if any neurologic changes beyond occasional mental retardation The disease still advances more rapidly than in

adults and death may occur a few years after onset although most child hood cases survive into adult life

In the adult the majority of the slowly developing clinical manifestations are related solely to the effects of an increasing mass of Gaucher cells in the bone marrow spleen liver and lymph nodes and occasionally a few other tissues The typical case is often diagnosed when asymptomatic splenic enlargement is detected during a routine examination In time the continuing enlargement of the spleen leads to distressing pressure symptoms and liver enlargement and moderate lymphadenopathy will have been noted Concomitantly increasing pigmentation of the skin and frequently pigmented areas of scleral thickening (pingueculae) appear in the eyes Bone lesions may give rise to pathologic fractures and the additive effects of marrow and splenic involvement lead to significant pancytopenia necessitating plenectomy Eventually marrow replacement by Gaucher cells may preclude adequate blood cell formation and as liver and lymphatic involvement increase there is general deterioration in nutritional state and death re ults from intercurrent infection The natural history of Gaucher s disease will no doubt be altered by the increasing use of antibiotics and the survival of both children and adults improved

### THE GAUCHER CELL

The typical cell in Gaucher tissues is a unique pathologic entity its presence makes possible an unmistakable diagnosis The peculiar feature of these large (20 to 100 microns) cells is the reticular pattern in the cytoplasm resembling nothing so much as wrinkled tissue paper or crumpled silk (Fig 19 1) This irregular streaked appearance is most obvious in fixed preparations stained with Mallory s trichrome connective ti ue stain and is e pecially well demonstrated by pha e microscopy [8] Strong acid phosphatase activity is demonstrable histochemically [23a] The many studies relative to the cytology and histogenesis of the Gaucher cell have been comprehensively reviewed by Pittaluga and Goyanes [27] and Block and Jacobson [28]

It is believed that the Gaucher cells arise from the reticulum cells [3 10 29–33] and possibly osteoblasts and fibroblast like spindle cells adjacent to bone spicules in the marrow [28] The absence of reactivity with the usual fat tains and the strong staining by the periodic acid-Schiff reaction [34–37] lend support to the generally accepted assumption that the cells are filled with cerebro ide The PAS po itive material is difficult to extract with lipid solvents [34–37] a phenomenon which has been interpreted as strong (protein) binding of the lipid within the cells [37] Using supravital techniques Erf tudied Gaucher cells in various tages of development and reported the gradual tran formation of the cytopla m of the cells from a liquid to a semisolid tate as presumably

cerebrosides accumulated [33] As the cells matured the mitochondria disappeared The cells are seldom seen in mitosis and appear to divide mainly by nuclear cleavage [27 39] Attempts to produce Gaucher cells by injection of cerebroside suspensions have been made [34 40–44] and transient changes in reticulum cells having some of the typical features have been observed None of the findings necessarily has direct bearing on the etiology of Gaucher's disease Granulomatous changes do not

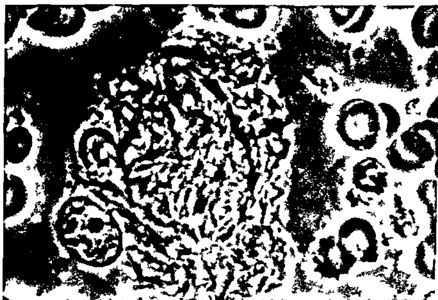


Fig 19 1 The Gaucher cell shown in supravital preparation of bone marrow biopsy The size of the cell may be compared with that of the adjacent erythrocytes (*Photomicrograph courtesy of Dr George Brecher National Institutes of Health*)

accompany Gaucher cell formation and histologic evidence of another primary process is absent

The transformation of reticulum cells to Gaucher cells produces striking changes in several organs and these with their attendant functional disturbances are worthy of comment

SPLEEN

Widespread replacement of the normal architecture of the spleen by Gaucher cells is characteristic and an enlarged firm nontender organ is palpable in nearly every case with a few notable exceptions [26 28 38 44] Although gigantic splenomegaly in the later stages may produce considerable mechanical distress perisplenitis and rupture are uncommon The most important consequences are thrombocytopenia leukopenia and anemia related to hypersplenism

The anemia is slight to moderate and usually increases with the duration of the disease Few erythrokinetic studies have been made although a shortened erythrocyte life span has been suggested [45] A moderate reticulocytosis is common [46] but presumably decreased erythropoiesis in the involved marrow as well as increased erythrocyte removal by the spleen both contribute to the anemia

A hemorrhagic tendency is a frequent complication in the adult case and may be the presenting sign That this is due to the frequent thrombocytopenia is indicated by the clotting studies which have been made The morphology of the megakaryocytes and platelets is normal and splenectomy promptly causes the platelet count to rise A rare case has been reported in which prothrombin time was increased associated with hypofibrinogenemia and deficits in convertin and accelerin [47] The tendency to spontaneous bleeding is expressed in numerous hemorrhagic infarcts and may account for some of the marked hemosiderosis in the tissues of the adult cases

LIVER

The liver is consistently and diffusely involved the masses of Gaucher cells appearing grossly as white translucent spots The pressure of these expanding masses destroys parenchymal cells and the lobular architecture is distorted with an accompanying increase in connective tissue Groups of cells may surround the central and hepatic veins and obstruct the liver capillaries [48-50] Moderate hepatomegaly is present in the majority of patients [46] and ascites may occasionally be present [49] There are no characteristic changes in liver function test results [46 50 51] or in serum protein concentrations [52] In a rare case the serum alkaline phosphatase level may be elevated in association with severe osseous involvement [53]

BONE

The development of abundant Gaucher cells—often peculiarly fascicular or spindle shaped—throughout the marrow and cancellous bone is common and may lead to spectacular nearly complete replacement of marrow accompanied by pain pathologic fractures and vertebral collapse [3] The destruction of bone and joint cartilage is accomplished through pressure or ischemic necrosis true invasion of cortex periosteum or cartilage has not been observed [3] The phalanges long bones vertebrae ribs or pelvis are involved much more commonly than the skull although mandibular lesions have been noted Expansion of the cortex at the lower end of the femur producing a radiolucent area with the contour of an Erlenmeyer flask is responsible for a characteristic deformity Destruction of the vertebral bodies produces a thin shortened cylinder often ending with complete spinal fusion and gibbus forma

tion Bone pain may be accompanied by erythema and swelling and sinuses with Gaucher cells in the drainage [53], may develop simulating osteomyelitis

Junghagen s thorough study published in 1926 contains one of the most extensive descriptions of the radiologic changes in the bones in Gaucher s disease [54] Relief of acute bone pain has been reported after x ray therapy, as has radiologic improvement in a large cystic lesion [55] Radiation has also failed to influence the development of a typical lesion in bone [8]

### LYMPHOID TISSUE

Gaucher cells commonly occupy the area of the general reticulum outside the lymph sinuses [30] in the deep abdominal and thoracic lymph nodes, they frequently cause enlargement of peripheral nodes as well Peripheral lymph gland enlargement is much more common in infantile than in adult cases [6 9] There is also present an increased accumulation of iron containing pigment in the nodes most being present in reticular macrophages but some unequivocally present in the Gaucher cells [27 30] Although the degree of lymph node involvement is quite variable biopsy of this tissue is useful for diagnosis

### EYES

Brill [56] in his early description of two involved siblings first called attention to the pingueculae in Gaucher s disease In about 25 per cent of patients there are present bilaterally on the sclera striking yellow brown wedge shaped patches whose bases abut the cornea with the apices extending to the canthi [46] They are asymptomatic and require no treatment

The pingueculae in Gaucher s disease may occur at any age beyond childhood and are larger and darker than those commonly seen in otherwise normal individuals after middle age In one instance [57] small collections of large epithelial cells with foamy cytoplasm which did not take conventional lipid stains have been described in a Gaucher pinguecula Typical Gaucher cells have not been described in these lesions

### SKIN

Characteristic diffuse yellow brown pigmentation develops on the exposed surfaces in many adult patients It is sometimes localized or unilateral [9] or it may be symmetrically distributed on the lower legs [58] or over the face associated with a characteristic malar flush Diminution of pigmentation following splenectomy has occasionally been reported [59] Definitive studies on the pathogenesis of the pigmentation are

needed Gaucher cells have not been found in the skin although in creased iron containing pigment [58] and melanin [60] have been reported

### OTHER ORGANS

As previously mentioned infantile patients may show heavy Gaucher cell infiltration in tissues other than spleen liver lymph nodes and bone marrow Heavy involvement of the lung may be present producing miliary opacities in the pulmonary x ray [7] Changes in the lung paren chyma with Gaucher cells in the sputum in one case have been reported in two adults [61] Rarely in adults the adrenal gland [62] thyroid [29] and kidney [63] may also show histologic lesions without evidence of functional impairment

### NEUROLOGIC LESIONS

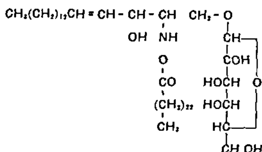
Present knowledge of the nature of the process occurring in the brain of infantile cases and its relationship to altered neurologic function is highly inadequate The histologic changes reviewed by several authors [2 37 64–68] consist of (1) nonspecific alteration in the cytoplasm of the ganglion cells with loss of Nissl substance and sometimes [2 68] vac uolization and ballooning of the cell body and (2) glial proliferation Some evidence of demyelination has been reported [69] Only on rare occasions [69 70] have Gaucher cells been seen in the brain and the e were localized to the hypophysis and hypothalamus or to the perivascular areas There are two reported cases in children in which neurologic function was abnormal but no histologic changes could be found in the brain after careful search [2 71] Furthermore as discussed in more detail later the cerebroside content of the brain has never been demon trated to be elevated Also the cerebroside present is of the normal type as opposed to the altered cerebroside in the viscera It should be noted that while lesions in the brain are classically considered to be restricted to infants altered ganglion cells may be seen in an occasional adult patient [70] Exhaustive microchemical dissection' of the Gaucher brain is sorely needed

Before discussion of the chemical changes in Gaucher s disea e a review of what is known about cerebrosides and their metabolism may be helpful

## CEREBROSIDES

### STRUCTURE AND OCCURRENCE

Cerebrosides consist of ceramide (N acyl phingo ine) and hexo e The mol r ratios of phingo ine base fatty acid and hexose are 1 1 1 as indicated by the accomp nying structural formula




N lignoceryl O[1] galactosyl sphingosine kerasin

These compounds were first isolated from brain and given the name cerebrosides by their discoverer, Thudichum [20]. Although Thudichum described their general structure correctly, it is through the subsequent work of a number of other workers including notably Thierfelder, Levene, Rosenheim and Klenk that the nature of the fatty acid and hexose moieties has been gradually understood.

A number of other generic names for these compounds has been used, such as galactolipids, galactosides, glycolipids (for glucocerebrosides), sphingoglycosides and glycosphingosides. None of these is more useful in distinguishing the compounds having sphingosine, fatty acid and hexose in equimolar amounts from other sugar-containing sphingolipids found in tissues (see below). The use of trivial names for various cerebrosides based on the nature of the fatty acid component, e.g., cerebron for the compound containing cerebronic acid, has long been a common practice. It is becoming less frequent, largely because of increasing awareness of the complexity of the fatty acids present in tissue cerebrosides and supposedly pure preparations of a given compound.

*Sphingosine Moiety*

The chemistry and biochemistry of sphingosine base have been discussed previously (Chap. 17). Many of the studies of sphingosine structure have utilized base isolated from brain and spinal cord cerebrosides. It is established with near certainty that in naturally occurring cerebrosides the $C_4$–$C_5$ double bond of sphingosine has the *trans* configuration [72, 73] and the substitutions at $C_2$ and $C_3$ the *erythro* configuration [74, 75]. A large fraction of the base in naturally occurring cerebrosides is the saturated compound dihydrosphingosine [76].

*Fatty Acid*

The fatty acid joined to sphingosine in acid amide linkage in cerebrosides is of variable composition and has been the subject of intensive research and lively debate since the discovery of these compounds. Fortunately a mist of confusing terminology generated by these activities

has largely disappeared [1] and the recent application of gas chromatography and improved techniques for separating cerebrosides has greatly increased available information in this area

By 1930 it was well established that the major acids in brain cerebrosides contained 24 carbon chains The first two cerebrosides isolated by Thudichum kerasin and phrenosin (cerebron [80]) were found to contain primarily lignoceric acid [$CH_3(CH_2)_{22}COOH$] [81–83] and phrenosinic (cerebronic) acid [$CH_3(CH_2)_{21}CHOHCOOH$] [84 85] respectively To these were subsequently added nervon containing nervonic acid [$CH_3(CH_2)_7CH{=}CH(CH_2)_{13}COOH$] [86] and oxynervon containing oxynervonic acid [$CH_3(CH_2)_7CH{=}CH(CH_2)_{12}CHOHCOOH$] [85] It was soon established that the most important acid was probably cerebronic [84]

It subsequently became clear however that the quantitative estimates of the content of particular cerebrosides might be in error because of preferential solubilities introduced by the techniques of isolation and *that many homologues of the $C_{24}$ acids are present varying in chain* length from at least $C_{16}$ to $C_{26}$ [78 87–93]

The studies of Kishimoto and Radin [93 94] are the most complete of the available analyses of the fatty acid components They have demonstrated that rat brain cerebrosides contain a broad spectrum of acids The saturated $\alpha$ hydroxy acids constitute the major component In this group the $C_{24}$ acids predominate but $C_{23}$ and $C_{22}$ acids are also present in appreciable amounts The saturated normal acids (mainly $C_{24}$ then $C_{22}$) are next in order of concentration The unsaturated acids both normal and hydroxylated and again mainly $C_{24}$ in length are minor components

The relative concentrations of all these classes of cerebroside fatty acids vary with maturation of the brain The major change in postnatal development of the rat brain is an increase in the saturated hydroxy acids associated with a fall in the unsaturated acids [94]

### *Hexose*

The hexose in brain cerebrosides called cerebrose by Thudichum was first identified as D galactose by Thierfelder [95] and Brown and Morris [96] All subsequent analyses have indicated that practically all (>90 per cent) of the hexose in the cerebrosides contained in a variety of normal tissues from several species is galactose [22 97–100] Small amounts of glucose have been reported in normal spleen and brain cerebrosides [illegible 98 101] The hexose present in cerebrosides isolated from the viscera in Gaucher's disease is significantly different from that present in normal tissues (see below)

[1] For the reader interested in the historical development of cerebroside chemistry several other references are suggested [illegible – 9]

The hexose is linked to sphingosine through the C 1 hydroxyl [102 103] Since cerebroside is not a reducing substance the linkage includes the aldehydic carbon of the sugar It has not yet been established whether the glycosidic linkage is through an $\alpha$ or $\beta$ anomeric bond Psychosine (galactosphingosine) prepared from normal brain glycolipid [104] and glucocerebroside from Gaucher spleen [22] have been hydrolyzed by $\beta$ galactosidase (emulsin) Others have found enzymatic reactions favoring an $\alpha$ linkage [105] Spectrophotometric evidence for the $\beta$ linkage in Gaucher glucocerebroside has also been recently reported [106]

## PROPERTIES AND ISOLATION

Cerebrosides are alcohol soluble acetone insoluble optically active compounds which can be crystallized in a relatively pure state Their determination requires lipid extraction with careful purification followed by hydrolysis and quantitative estimation of the sugar moiety Quantitative methods for cerebroside determination have been recently reviewed in detail by several authors [107–109] Chromatographic techniques for purification have also been developed [93, 107 109 110]

The major problem in analysis of cerebrosides is their isolation from other glycolipids These latter include not only gangliosides which are distinguished by their content of hexosamine and sialic acid (Chap 17) but other neutral glycolipids which contain more than one mole of hexose per molecule Klenk and coworkers [97 111] have isolated cerebroside-like molecules from both beef spleen and human erythrocytes which contain about 40 per cent hexose including both galactose and glucose Klenk [100] has noted that these appear to be transition compounds lying between gangliosides and cerebrosides and that they may signify an important pathway of interconversion between the several classes of glycolipids

Uzman and Rumley [112] have studied changes in the content of cerebrosides and water soluble glycolipids in the developing mouse brain The hexose and neuraminic acid contents of the latter compounds undergo changes during development at a time when cerebrosides are actively being laid down in the brain These changes are also interpreted by the authors as suggesting a precursor role for water soluble glycolipids in cerebroside formation In this regard further characterization of the ceramide portion of such lipids is needed Klenk and Rennkamp [97] found in bovine tissues that the fatty acids in gangliosides and cerebrosides were similar in the spleen but dissimilar in the brain

## CEREBROSIDE CONTENT OF TISSUES

The difficulties in obtaining pure cerebrosides have led to conflicting and sometimes meaningless reports of ' cerebroside content of various

tissues The methodology must be carefully scrutinized in interpreting these data particularly those found in the older literature

In the brain the problem of separate determination of cerebrosides is of special importance because of the high content of gangliosides and other water soluble glycolipids The values obtained up to 1956 for cerebroside content of brain have been summarized by Edgar [108] A content of about 5 0 gm per 100 gm dry weight has been reported for human gray matter [113–115] and adult whole brain [116] and three times this concentration in white matter [113–115]

These estimates of cortical cerebroside content are probably too high More recent analyses indicate that cerebrosides are present in very low concentration in gray matter [117] Cerebroside is one of the characteristic lipids of the myelin sheath therefore not only is it present mainly in white matter but its content increases as myelination proceeds in maturation of the brain [94 112 115 118–121] In a careful study of the glycolipid content of mouse brain combined with histochemical estimate of myelin content Uzman and Rumley [112] found neither myelin nor cerebroside present at birth Cerebrosides became detectable at 10 days of age and then rose very sharply in concentration reaching a maximum in animals about 42 days old Similar findings have also been reported for the rabbit brain [121] Differences with age in the cerebroside content of brain are also indicated by studies in the rat by Kishimoto and Radin [94] who found the content to rise from 0 34 gm per 100 gm wet weight of tissue to 1 1 gm in animals approximately 23 and 418 days old respectively Less precise figures for changes with maturation are available for the human brain The content of cerebrosides has been reported to increase until about age 16 after which it declines slightly [120]

In non nervous tissue the concentration of cerebrosides is much lower The highest concentrations in viscera (on the order of 0 5 gm per 100 gm dry weight) are found in lung and spleen [116] Many values for the concentration of cerebrosides in plasma have been reported In recent careful analyses Svennerholm and Svennerholm [122] found normal values to range from 3 5 to 5 7 mg per 100 ml The content of gangliosides was slightly lower According to Burt and Rossiter [123] the cerebroside content of leukocytes (rabbit) is considerably higher than that of erythrocytes The latter contain about 30 mg cerebroside (neutral glycolipid) per 100 gm fresh tissue

## BIOSYNTHESIS OF CEREBROSIDES

Relatively few studies of cerebroside biosynthesis have been made and these have been mainly confined to brain In this tissue following the intraperitoneal injection of the substrate the radioactive carbon atoms from acetate 1 $C^{14}$ and octanoate 1 $C^{14}$ [110 124] galactose 1 $C^{14}$ [125–128] glucose U $C^{14}$ [125 127–129] and glucose 6-$C^{14}$ [128 129] have been

found in neutral glycolipids (in most cases demonstrated beyond reasonable doubt to be cerebrosides) Such incorporation in vivo has been demonstrated in several species, including the monkey rat and mouse

It is also established that either glucose or galactose is mainly incorporated into the cerebrosides as a unit with most of the labeled glucose appearing as galactose in the cerebrosides [*127–129*]

The rate of incorporation of either galactose or glucose into brain cerebroside depends on age and apparently *is directly related to the rate of* myelin formation In mice some synthesis is demonstrable at birth the maximum occurring at about 16 to 22 days [*128 129*] or very close to the time of maximum myelin formation suggested by chemical analyses during maturation in this species [*112–119*] In rats there is practically no synthesis up to 7 days The maximum rate of incorporation of hexose is reached at 14 to 16 days and is reduced shortly thereafter [*127*]

When labeled hexose is injected intraperitoneally into young rats there follows a rapid rise in the activity in brain cerebrosides the maximum being reached in about 6 hr [*127*] or slightly later [*126*] The radioactivity then declines indicating active turnover of cerebrosides In the 15 day-old rats Burton et al [*127*] noted that the cerebroside radioactivity fell to a third of the maximum value within 24 hr Radin et al [*126*] found that the rate of incorporation of labeled galacto e into brain gangliosides was about the same as that for cerebrosides The cerebroside sulfatides were labeled more slowly

It has been recently shown by similar techniques that the maximum rate of synthesis of brain gangliosides is achieved at an earlier age than that for cerebrosides in rats [*130*] This is consistent with the earlier appearance of ganglioside in the brain and possibly with a precursor role of these compounds in cerebroside formation Moser and Karnovsky [*128*] have followed the incorporation of labeled glucose and galacto e into both cerebrosides and gangliosides in mice at different ages Cerebroside synthesis' appears to slack off with increasing age more pronouncedly than "synthesis of gangliosides The specific activity of galactose in cerebrosides moreover was greater than that in gangliosides As they point out it is extremely difficult to interpret such findings in terms of possible precursor product relationships They do not provide support however for the conclusion that the ganglioside fraction as a whole forms a precursor pool for the formation of cerebrosides

It is interesting that the incorporation of labeled hexose into brain cerebroside sulfatides is not associated with any appreciable rate of decay These substances increase during much of the life span [*118*] and may have important implications in the processes of aging in the central nervous system [*126*]

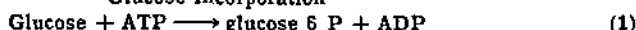
### STUDIES IN VITRO

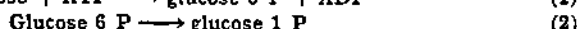
Definitive studies of the synthesis of cerebrosides in cell free brain tissue have been made by Burton Sodd and Brady [*127*] All subcellular brain fractions are capable of incorporating galactose $C^{14}$ into cerebrosides the greatest activity being associated with the microsomal fractions It is proposed that the pathways for both glucose and galactose incorporation into brain glycolipids involve the uridine nucleotides [*127 131*] with prior conversion of glucose to galactose [*127*]

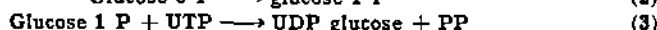
The glycosyl transfer reactions have been described in detail in Chap 7 All the necessary enzymes have been demonstrated in brain [*132–136*]

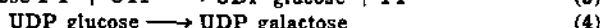
For incorporation of galactose and glucose into cerebrosides the reactions as summarized by Burton et al [*127*] include

**Glucose Incorporation**


Glucose + ATP ⟶ glucose 6 P + ADP (1)

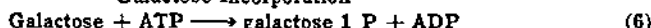

Glucose 6 P ⟶ glucose 1 P (2)


Glucose 1 P + UTP ⟶ UDP glucose + PP (3)


UDP glucose ⟶ UDP galactose (4)


UDP galactose + acceptor ⟶ cerebroside + UDP (5)

**Galactose Incorporation**


Galactose + ATP ⟶ galactose 1 P + ADP (6)


Galactose 1 P + UDP glucose ⟶ UDP galactose + glucose 1 P (7)


UDP galactose + acceptor ⟶ cerebroside + UDP (5)

The position of UDP galactose as immediate donor (Reaction 5) is demonstrated by the failure of either galactose 1 P or galactose + ATP to be utilized unless UDP gluco e is also present The conversion of glucose to galactose before incorporation is strongly suggested by the inhibition of utilization of glucose but not of galactose when the cell free particles are preheated UDP galactose 4-epimerase catalyzing Reaction 4 is heat sensitive Chromatographic evidence was obtained in these studies that the cerebrosides synthesized included N lignoceryl $O^1$ and N-cerebronyl $O^1$ galactosyl sphingosine [*127*]

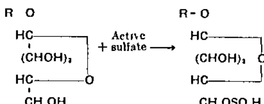
In Reaction 5 the acceptor for the hexose is indicated to be ceramide but this has not been proved Other evidence has been presented that the acceptor may be free sphingosine [*128 131a*] It should not be concluded that conversion of ceramide or sphingosine to cerebrosides through the uridine nucleotide pathway is neces arily the only or even the major route of cerebroside synthesis in all organs It should al o be noted that some glucose can apparently be incorporated directly into cerebrosides [*128 129*]

Trams and Brady have demon trated incorporation of both labeled galactose and gluco e into cerebrosides by human spleen tissue slices obtained from a patient with Gaucher disease and another with Nie

mann Pick disease [137] Rat liver apparently also may incorporate a very small amount of hexose into cerebroside [127]

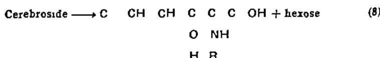
DEGRADATION AND UTILIZATION

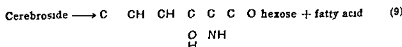
Several theoretic pathways exist for cerebroside degradation or further utilization One is conversion to cerebroside sulfatides




The lack of evidence for appreciable turnover of the sulphatides suggests that this would be mainly a unidirectional reaction [126] In this regard it may be significant that the ratio of cerebro ide sulfate to cerebrosides increases with age in the brain [94] Other possible reactions are conversion to polycerebrosides ( ee below) or other water soluble glycolipids through further addition of hexose hexosamine and sialic acid units None of these reactions has been demonstrated and speculations that reactions in the opposite direction might be more important [100 112] have been previously mentioned

Cerebrosides may be degraded to (1) ceramide and hexose through hydrolysis of the glycoside bond (Reaction 8) and in theory to (2) psychosine and fatty acid through hydrolysis of the acid amide bond (Reaction 9)




The first reaction has been demonstrated in tissues the second has not It has been reported that small amounts of ceramide may be present in liver and spleen [138 139] but neither free ceramide nor psychosine has been reported in brain

The production of ceramide (Reaction 8) is catalyzed by several types of hexoside hydrolases Galactosidases such as are found in sweet almond emulsin [22] and extracts of *Escherichia coli* [105] split cerebrosides A "cerebrosidase was first discovered in extracts of human spleen brain

and pancreas by Thannhauser and Reichel [140] They found that hydrolytic activity was greatly enhanced by activation of the extract with reducing substances such as hydrogen sulfide cysteine or reduced glutathione Their work has been confirmed by Fujino [141] who determined that the products of the reaction were ceramide and hexose Under the conditions employed the splenic extracts did not attack the N acyl linkage of ceramide No enzymatic hydrolysis of this bond has been studied Other evidence suggestive of cerebroside-splitting activity in nervous tissue is the disappearance of glycolipid which occurs during Wallerian degeneration [142] It has been observed that this process is accompanied by an increase in macrophages or Schwann cells about the affected peripheral nerve suggesting that these cells may contain the degradative activity [143]

Thus far too little is known of the manner in which cerebrosides are broken down or converted to other products Although ceramide has been repeatedly discussed as a pivotal intermediate in sphingolipid synthesis [140] there have been no studies on relatively pure enzymes which reveal the equilibriums and other conditions by which ceramide might be preferentially used for one or another sphingolipid

## CHEMICAL ABNORMALITIES IN GAUCHER'S DISEASE

### TISSUE CEREBROSIDES

The characteristic chemical alterations in Gaucher tissues involve the cerebroside content The alterations have two possibly three important features These are (1) an increase in total cerebroside concentration (2) accumulation of cerebrosides containing abnormal sugar components and (3) possible alterations in the physical state and molecular form of the cerebrosides Significant alteration in the content of other tissue lipids has not been demonstrated

#### *Cerebroside Content of Tissues*

In involved viscera the cerebroside concentration may be increased by tenfold or more [9 65 87 116] Extensive analyses of a case published by Ottenstein Schmidt and Thannhauser [116] and by Thannhauser [9] are shown in Table 19 1 The few quantitative analyses of brain tissue in which there were anatomic abnormalities [87 116] do not demonstrate any increase in cerebroside content although an increased content of polycerebrosides in a Gaucher brain has been reported by Uzman (see below)

#### *Carbohydrate Content of Cerebrosides*

The cerebrosides in the viscera in Gaucher's disease contain mainly glucose rather than the galactose found in normal cerebrosides

Whether an increase in galactocerebroside also occurs in this disease has been seriously questioned [98] but it should probably be accepted that it does. In Table 19.2 are summarized the reports in which some attempt was made to identify the cerebroside carbohydrate. The earlier studies relied mainly on the melting point of the osazone and fermentation studies. Differential color reactions have been frequently used and more recently paper chromatography and specific enzymatic reactions such as glucose oxidase [106].

TABLE 19.1 CEREBROSIDE CONTENT IN ORGANS FROM A 3½ MONTH OLD PATIENT WITH GAUCHER'S DISEASE

| Organ | Total cerebroside content mg per 100 gm dry weight | |
|---|---|---|
| | Gaucher's | Normal |
| Spleen | 1.44 | 0.1–0.5 |
| Liver | 0.33 | 0.05–0.15 |
| Lung | 1.01 | 0.1–0.6 |
| Kidney | 0.54 | 0.1–0.7 |
| Pancreas | 0.14 | 0.1–0.3 |
| Thymus | 0.60 | |
| Adrenal gland | 0.[illegible] | |
| Brain | 3.2[illegible] | 4.0–[illegible].0 |

Source: Abstracted from the data of B. Ottenstein et al. [116], also published by S. J. Thannhauser [9]. The normal values for brain were those obtained in a 1-day-old child and are undoubtedly lower than normal for the age of the patient with Gaucher's disease.

The analyses reported up to 1950 were compiled and critically reviewed by Brante [98]. He also reported 4 cases in which glucose represented over 90 per cent of the sugar in the splenic cerebrosides. Brante further concluded that in the instances where galactose was reported as the major sugar [16, 116, 144, 145] the methods may have been subject to error and that any increase in galactocerebrosides was unproved. At least 12 more case studies have been subsequently reported. In 8 of these the spleen cerebrosides contained practically only glucose. There are several important exceptions however. Montreuil et al. [101], using paper chromatographic methods capable of separating glucose and galactose mixtures, found 45 per cent galactose in the cerebrosides. Larke [155] found equimolar concentrations of glucose and galactose after complete hydrolysis and lactose after partial hydrolysis. Woolf [156], who found mainly glucose after complete hydrolysis, also reported finding paper chromatographic evidence of fructose, lactose and sucrose after only partial hydrolysis. In the cerebrosides isolated in polymolecular form discussed below, Uzman found a preponderance of galactose. The

TABLE 19-2 THE NATURE OF CARBOHYDRATE IN CEREBROSIDES FROM GAUCHER TISSUES

| Case reference | Year | Tissue source | Cerebroside sugar | | |
|---|---|---|---|---|---|
| | | | Glucose | Galactose | Other |
| [16] | 19 4 | Spleen | | + | |
| [144] | 1933 | Spleen | | + | |
| [ 1] | 1934 | | + | | |
| [ ] | 1940 | Spleen | + (100%) | | |
| [87] | 1940 | Spleen | + (>95%) | | |
| [8 ] | 1940 | Brain | | + | |
| [8 ] | 1940 | Liver lung spleen | + (95%) | | |
| [1 5] | 1941 | Spleen | | + | |
| [1 6] | 1942 | Spleen | + (100%) | | |
| | | Liver | + (100%) | | |
| [1 7] | 1942 | Spleen | + | | |
| [1 8] | 1942 | Spleen | + | | |
| [149] | 194 | Spleen | + (100%) | | |
| [116] | 1948 | Spleen liver other viscera | + (major) | + (minor) | |
| | | Brain | | + (100%) | |
| [116] | 1948 | Liver spleen brain other viscera | | + (100%) | |
| [116] | 1948 | Spleen | + (100%) | | |
| [116] | 1948 | Spleen | + (100%) | | |
| [116] | 1948 | Spleen | + (45%) | + (55%) | |
| [116] | 1948 | Spleen | + (43%) | + (5 %) | |
| [116] | 1948 | Erythrocytes | | + (100%) | |
| [116] | 1948 | Erythrocytes | | + (100%) | |
| [150] | 1948 | Spleen | + | | |
| [1 1] | 1950 | Spleen | + ( 5%) | | |
| | | Brain | | + ( 5%) | |
| [98] | 1951 | Spleen | + ( %) | | |
| | | Liver | + (8 %) | | |
| | | Erythrocytes | | + (100%) | |
| [98] | 19 1 | Spleen | + ( 0%) | | |
| [98] | 1951 | Spleen | + ( %) | | |
| [98] | 1951 | Spleen | + (100%) | | |
| [15 ] | 1951 | Spleen | | + | |
| [1 3] | 1953 | Brain | | + | |
| | | Spleen | + ( 0%) | + (80%) | |
| [101] | 1953 | Spleen | + ( %) | + (1 %) | |
| [15 ] | 1954 | Spleen | + (100%) | | |
| [15 ] | 19 4 | Spleen | + (100%) | | |
| [155] | 19 4 | Spleen | + ( 0%) | + (50%) | Lactose (after partial hydrolysis) |
| [156] | 1954 | Spleen | + (> 0%) | + | Fructose (trace) lactose and sucrose (after partial hydrolysis) |
| [156] | 1954 | Spleen | + (> 0%) | + | |
| [156] | 1954 | Spleen | + | | Fructose (trace) sucrose fructose galactose (after partial hydrolysis) |
| [15 ] | 19 8 | Spleen | + (100%) | | |
| [10 ] | 19 8 | Spleen | + (100 ) | | |

Sugar determined after isolation as Gaucher lipoprotein [15 ] or polycerebroside [153]

reported differences in carbohydrate moiety have no correlation with the age of the patients

In the case reports of Ottenstein et al [116] the question of galactocerebroside identification is especially important These authors who used fermentation in addition to the o azone for identification found increased glucocerebrosides in one case and increased galactocerebrosides in an involved sibling If they are not due to subtle errors in experimental technique these findings and those indicating polysaccharides in the Gaucher cerebrosides tend to diminish h the implications of a specifically abnormal compound in the involved tissues

In viscera in Gaucher s disease nevertheless the usual substitution of glucose for galactose in the excessive cerebrosides may constitute a key biochemical abnormality The interesting findings of disaccharide need further evaluation It is important in this regard that most of the cerebrosides carefully i olated from Gaucher tissues have contained enough *reducing substance (after complete hydrolysis) to account for only* a single hexose unit in the molecule

The brain cerebrosides examined so far have contained galactose only

*Polymolecular Substances*

Uzman has reported several interesting studies of the form in which cerebrosides may be present in the Gaucher spleen and brain [152 153] These include the isolation of the cerebrosides in a "Gaucher lipoprotein and by another approach as polycerebroside"

In the first instance spleen was extracted in aqueous media and after repeated precipitation and re solution 70 per cent of the total cerebroside was retained in solution in saline This water soluble material contained about 40 per cent protein and 60 per cent lipid almost all of which was cerebroside No lipid phosphorus or free amino nitrogen was present A molecular weight for this lipoprotein obtained by osmometry was about 320 000 As estimated from the difficulty with which the protein was freed of lipid by solvent extraction the lipid protein binding was quite strong No comment was made about the possible presence of the lipoprotein in normal tissues

In the second study [153] water soluble glycolipid was isolated from *Gaucher spleen and brain This material was more soluble in acidified* aqueous media than ordinary cerebroside but it did not contain the hexosamine or sialic acid characteristic of ganglioside or ' mucolipid ' (Chap 17) Cerebrosides were liberated by hydrolysis and these contained a galactose to glucose ratio of about 4 1 This polycerebroside migrated on electrophoresis as a single boundary with a negative charge The absence of phosphorus or sulfate suggested this charge may have been due to free carboxyl groups The molecular weight was estimated by diffusion and osmometry to be about 30 000

Uzman reported finding polycerebroside in normal brain in small quantities but not in the spleen in the absence of Gaucher s disease [153] He suggested that polycerebroside was not necessarily a polymer of repeating cerebroside units and that it could be a precursor for the Gaucher lipoprotein which he had earlier described

Confirmation and extension of these interesting findings are awaited Klenk in reviewing his studies of the Gaucher spleen [100] states that the water soluble polycerebrosides described by Uzman could be isolated in only small amounts (46–176 mg per 100 gm of wet tissue ) He makes the further observation that the polycerebrosides resemble the glycolipids in bovine spleen [97] and suggests that the Gaucher cerebrosides might be liberated enzymatically from water soluble glycolipids like polycerebrosides and gangliosides

Other possible abnormalities in cerebroside structure such as in the sphingosine or fatty acid portions of the molecule have not been established in Gaucher s disease Behenic acid (saturated $C_{22}$) has been twice reported as a major component of the excessive cerebroside in the spleen liver and lung [87 106] In the brain of one of these cases an infant with ganglion and glial cell alterations the major fatty acid in the brain cerebrosides was cerebronic This was interpreted by Klenk [87] as suggesting that in Gaucher s disease the involved tissues contained cerebroside with the fatty acid moiety normally characteristic for that tissue [2]

*Acid Phosphatase*

Striking increase in acid phosphatase activity in plasma and tissues is now a well established phenomenon in Gaucher s disease Tuchman et al [25] were the first to report increased activity in plasma noted as a chance observation They subsequently investigated 17 more cases and found in every one increased activity against phenylphosphate in acid medium (7 to 14 Gutman units as compared with the normal range of 4 to 5 units) [158] When β glycerophosphate was employed as substrate (Bodansky method) activities were lower and many were within normal limits In contrast to the prostatic enzyme [159] the plasma acid phosphatase in Gaucher s disease is not inhibited by L-tartrate [158] Neither is it inhibited significantly by copper ions or by formaldehyde as is the normal erythrocyte phosphatase [159]

*Crocker and Landing* [23a] have recently reported extensive studies

[2] Marinetti et al [1 4] have recently published detailed analyses of cerebroside isolated from the spleen of a 50-year-old patient with Gaucher s disease The cerebroside concentration was 0 89 gm per 100 gm of fresh tissue Glucose appeared to be the only sugar present The hexose was shown to be attached to the primary hydroxyl group of trans sphingosine As determined by gas chromatography the major fatty acids were lignoceric behenic and palmitic

initiated years ago with the observation by Farber of marked acid phosphatase activity in the Gaucher cells themselves All histiocytic cells have some weak acid phosphatase activity but the degree of hyper phosphatasia present in Gaucher tissues is not found in other conditions associated with enlarged macrophages such as Niemann Pick disease [23a] With the possible exception of a single case [160] the plasma phosphatase is also normal in Niemann Pick disease (Chap 18) It should be noted that one metabolic disease of bone osteopetrosis is also associated with acid hyperphosphatasia [161 23a]

Crocker and Landing [23a] are of the opinion that the increased enzyme activity in plasma has its origin in the Gaucher cells it is not dependent upon the presence of the spleen They also believe that the hyperphosphatasia may be an important clue to the etiology of the disease Splenic acid phosphatase has been found active against a large variety of substrates with maximum activity against nucleoside triphosphates such as ATP [23a]

*Blood Lipids*

There are no characteristic changes in the serum lipids in Gaucher s disease Most importantly no increase in cerebrosides has been found Thannhauser, using the methods developed by Svennerholm and Svennerholm [122] found 1 83 mg cerebroside per 100 ml plasma in a normal male and 1 23 and 1 83 mg per 100 ml in two adults with Gaucher s disease [9] An increase in lipid nitrogen was reported in several cases by Bloem et al [58] but this material was not further characterized The nature of the hexose in the circulating cerebrosides in Gaucher s disease has not been investigated

## ETIOLOGIC POSSIBILITIES

It is all too apparent that present knowledge about normal glycolipid metabolism is inadequate to bear the burden of excessive speculation about the basic abnormality in Gaucher s disease There may even be more than one biochemical lesion of importance Several aspects of the disease deserve further comment with respect to etiology

### TRANSPORT OF CEREBROSIDES

It is most probable as Thannhauser was among the first to emphasize [9] that the excessive cerebrosides are made by the Gaucher cells or their normal appearing neighboring reticulum cells The normal concentration of cerebrosides in extracellular fluid offers no evidence of increased trans port from a remote site in this manner It cannot be denied however that increased erythrocyte breakdown might account for considerable of the cerebrosides collecting in reticuloendothelial tissues This was early

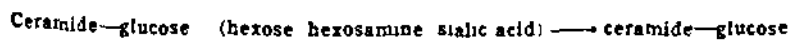
suggested by Marchand [12] and is supported by the accumulation of hemosiderin adjacent to and in the Gaucher cells and by the clinical impression that splenectomy results in improvement in some parameters other than hematologic ones To be sure the absence of Gaucher cells in other types of hemolytic anemias the paucity of hemosiderin in the tissues in infantile cases and above all the presence of abnormal cerebrosides in the viscera mitigate against the primary importance of this mechanism The latter objection may not prove to be valid however when more information about the glycolipid content of normal and *Gaucher* erythrocytes is obtained

## DEFECTIVE CEREBROSIDE METABOLISM

The appearance of glucose in the Gaucher cerebrosides found in the viscera is undoubtedly a most important clue The finding of Trams and Brady [137] that a Gaucher spleen was capable of incorporating either galactose or glucose into cerebrosides in vitro is significant if not too surprising There is no clinical evidence of a general defect in either galactose or glucose metabolism such as that in galactosemia although the individual glycosyl transfer reactions might well be studied in Gaucher tissues More data will need to be compiled concerning the relative rates of hexose utilization in normal and involved tissues [137]

Whatever the status of glycolipid synthesis in Gaucher s disease it is evident that there is some step in the degradation or elimination of cerebrosides which is disastrously rate-limiting Beyond the previously mentioned studies with crude preparations of cerebrosidase the pathways for utilizing cerebrosides are unknown It would be interesting also to know the ability of the normal complement of dissimulative enzyme to utilize glucocerebrosides as compared with galactocerebrosides

The suggestion *of abnormal polycerebrosides in Gaucher tissues and* the possible interrelationships between the several classes of glycolipids which have been suggested [100 112 153] also raise an intriguing possibility demanding much further work This is the accumulation of glucocerebrosides as end products of excessive synthesis and degradation of gangliosides or the more complex neutral glycolipids by the following type of reaction


Ceramide—glucose (hexose hexosamine sialic acid) ——→ ceramide—glucose

The relationship of such a mechanism to the possible presence of protein having an abnormally high affinity for cerebrosides [102] also requires clarification

The possibility *that the hyperphosphatasia characteristic of Gaucher* tissues might lead to degradation of phospholipid with diversion of substrate to cerebrosides has been considered but left unsupported by the

finding of normal amounts of phospholipid in these tissues [23a] Future work may supply a key to etiology by disclosing a more subtle effect of the phosphatase on cerebroside metabolism

Finally no aspect of Gaucher s disease is less well understood than the changes in the nervous system The dichotomy between the types of cerebrosides found in the brain and viscera the lack of good evidence for any remarkable degree of abnormal myelination although a general defect in cerebroside metabolism is supposedly present and the reason for the changes in the ganglion cells will all no doubt become clear when more of the normal metabolic pathways are unraveled

## DIAGNOSIS

The diagnosis of Gaucher s disease can be made for practical purposes by the finding of the typical cells in spleen liver bone marrow lymph node or other tissues The relative merits of various tissues as a biopsy site have been discussed at length by Block and Jacobson [28] As discussed below there may be a "carrier' state of the disease in which the finding of immature appearing cells may be the only abnormality

Although the finding of Gaucher cells leaves little doubt concerning the diagnosis in every case attempt should be made to ascertain an elevation of cerebrosides in the involved tissues and to determine chemically the nature of the cerebrosides present A serum acid phosphatase determination should also be made using L-tartrate inhibition or similar means to differentiate the enzyme activity from the prostatic enzyme

The characteristic nature of the skin pigmentation pingueculae and x ray changes in bones combined with other clinical features may help form a presumptive diagnosis but it should always be supported by histologic and chemical studies

## TREATMENT

There is no specific treatment Splenectomy must often be performed because of mechanical discomfort or the hematologic effects of hypersplenism It is followed by a prompt rise in platelets and leukocytes which may persist for years The increase in red cell mass which also occurs is often transient [9] Occasional improvement in pigmentation and decrease in pingueculae have been reported after splenectomy [59] Cortisone may reduce capillary fragility or induce a slight rise in platelet count preoperatively [163]

Irradiation of the spleen is of no value in massive splenomegaly [9] It is of great value in reducing bone pain [9] Its use must be carefully tempered by consideration of the effect it will also have on the surviving bone marrow Bone pain may also respond well to steroid therapy [9]

Gauchers disease is not incompatible with successful pregnancy [163 164] An increase in abortions and stillbirths has been considered typical of the di ease [165]

## GENETICS

The occurrence of Gaucher s disease in siblings was ob erved in the second case report to appear in the literature 1895 [24] Since that time ample evidence has accumulated to indicate that the condition is familial Sibships in which five of six [166] and four of five [6] members were involved have been reported

There is an apparent preponderance of Jews among reported cases although many have been reported in which Jewish ancestry was not present involving Caucasians Indians [167] Filipinos [168 169] Mexicans [170] Japanese [171] and Negroes [172] The sex distribution is about equal [4 165]

There is no present means of determining whether all reported cases of Gaucher s disease represent a single mutation causing an identical biochemical lesion There is some reason for suspecting they do not considering a recent review of the genetic aspects of some 110 cases by Hsia et al [173] They concluded that clinically Gaucher s disease may fall into at least two groups with different modes of transmission (1) autosomal recessive in the majority of cases and (2) autosomal dominant one of the few well detailed examples of which is presented by them Hsia et al also add a possible third type of mutation based on the observation that if neurologic abnormalities are present they appear in all siblings affected with Gaucher s di ease

Most familial cases which have been adequately described have shown a frequency of involvement lack of sex discrimination or consanguineousness try consistent with an auto omal reces ive transmi sion The most elaborate analysis of a pedigree of this type is that made by Herndon and Bender [172] of 5 cases in a closely related Negro ib hip in North Carolina These 5 cases had 17 siblings 11 definitely normal and 6 who died in infancy of unknown causes The apparent ratio of involvement in surviving siblings was therefore very clo e to 25 per cent The authors concluded that transmis ion as a dominant trait with reduced penetrance could be excluded and that there was no evidence for sex linkage

A dominant mode of inheritance was suggested by Groen [165] although in his review and 10 case pre entations there were no cases providing unequivocal support for this mode In 23 of 31 families Groen found that only one generation was involved he empha ized the relative rarity of vertical transmission He considered this to be due not only to decreased survival of patients into the reproductive period but also to increa ed frequency of stillbirths and abortions in marriages where

one partner has Gaucher s disease Groen suggested the possibility of anticipation or earlier development of the disease in succeeding generations until it extinguishes itself in infantile death Hsia et al [173] find the outlook for reproduction in involved families less gloomy Their analysis supporting several modes of transmission reinforces the need for determining the specific biochemical trait or traits and the determinants of their expression before the genetics of Gaucher s disease is clarified

## DETECTION OF THE TRAIT

A significant observation made by Groen has since been repeated by Stransky et al [168 169] This is the finding of small or 'young Gaucher cells in the marrow of a parent of an involved child Some of these parents have been noted to have had pingueculae or very slight splenomegaly but their general health has been good In each case a normal bone marrow biopsy was obtained in the opposite parent This possible means of detecting a carrier state in Gaucher s disease needs to be explored in families of all involved cases

Elevated acid phosphatase in plasma has not been found in relatives of patients with Gaucher s disease [23a 162] This test however should continue to be performed on relatives until its implications are established The possibility that erythrocyte cerebroside may be abnormal in carriers does not appear to have been explored The development of a better biochemical test for heterozygosity (assuming recessive inheritance) is urgently needed the ability to predict satisfactorily the hazard of transmission of this crippling disease would be of inestimable value

## SUMMARY

1 Gaucher s disease is a rare familial disorder characterized by accumulation of cerebrosides compounds containing sphingosine fatty acid and hexose in equimolar amounts in reticuloendothelial cells The storage cells possess a bizarre and characteristic appearance Their increasing mass is responsible for most of the clinical manifestations which include hepatosplenomegaly lymphadenopathy and bone lesions due to expansion of involved marrow

2 The disease may be detected at any age When it develops in infancy the course is malignant and further characterized by severe neurologic abnormalities These are associated with nonspecific changes in ganglion cells Cases developing in childhood may also lead to an early death or may progress slowly as in the adult form In the latter hematologic abnormalities associated with hypersplenism skin pigmentation and pingueculae usually develop

3 The involved tissues with exception of brain have a greatly in

creased content of cerebrosides In most cases the cerebrosides are abnormal in that they contain glucose instead of the usual galactose In several cases abnormal polycerebrosides or lipoprotein bound cerebrosides have been reported The biochemical basis for the increase in cerebrosides is not known

4 Elevated plasma acid phosphatase activity which is not inhibited by L-tartrate in contrast to the prostatic enzyme has been reported in all cases tested so far The etiologic implications of this finding are unknown

5 There is evidence that the disease may have several modes of transmission The majority of cases appear to be inherited as an autosomal recessive trait A few are consistent with autosomal dominant transmission The biochemical homogeneity of the responsible trait remains to be established

## BIBLIOGRAPHY


1 Gaucher P *De l'épithéloma primitif de la rate* Thèse de Paris 1882
2 Oberling C and Woringer P La maladie de Gaucher chez le nourisson Rev franç pédiat 3 475 1927
3 Pick L A classification of the diseases of lipoid metabolism and Gaucher's disease Am J M Sc 185 453 1933
4 Atkinson F B R Gaucher's disease in children Brit J Child Dis 35 1 1938
5 Klenk L Letterer L Eppinger H and Determan A Speicherkrankheiten Verhandl deutsch path Gesellsch 31 6 1938
6 Reich C Siefe M and Kessler B J Gaucher's disease a review and discussion of twenty cases Medicine 30 1 1951
7 Van Creveld S The lipidoses Advances Pediat 6 190 1953
8 Schettler G Cerebrosidose in *Handbuch der Inneren Medizin* vol 7 p 665 Springer Berlin 1955
9 Thannhauser S J *Lipidoses Diseases of the Intracellular Lipid Metabolism* Grune & Stratton New York 1958
10 Schlagenhaufer F Über meist familiär vorkommende histologisch charakteristische Splenomegalien (Typus Gaucher) Arch path Anat 187 125 1907
11 Bovaird D Jr Primary splenomegaly Am J M Sc 120 377 1900
12 Marchand F Über sog idiopathische Splenomegalie–Typus Gaucher München med Wchnschr 54 1102 1907
13 Pick L Zur pathologischen Anatomie des Morbus Gaucher Med Klin (Munich) 18 1408 1922
14 Epstein E Beitrag zur Chemie der Gaucherschen Krankheit Biochem Ztschr 145 398 1924
15 Epstein E and Lorenz K Die Phosphatidzellverfettung der Milz bei Niemann Picks hen Krankheit verglichen mit der Lipoidchemie des Morbus Gaucher und der Schüller Christianschen Krankheit Ztschr phys l Chem 192 145 1930
16 Lieb H Cerebrosidspeicherung bei Morbus Gaucher Ztschr physiol Chem 140 305 1924
17 Lieb H Cerebrosidspeicherung bei Splenomegalie Typus Gaucher Ztschr physiol Chem 170 60 1927
18 Lieb H and Mladenović M Cerebrosidspeicherung bei Morbus Gaucher Ztschr physiol Chem 181 208 1929
19 Thudichum J L W *Die chemische Konstitution des Gehirns des Menschen und der Tiere* Franz Pietzcker Tübingen 1901
20 Drabkin D L *Thudichum Chemist of the Brain* U of Penn Press Philadelphia 1958
21 Aghion A *La Maladie de Gaucher dans l'enfance* Thèse Paris 1934

22 Halliday N., Deuel H Jr., Tragerman L. J., and Ward W E. On isolation of glucose-containing cerebroside from spleen in a case of Gaucher's disease J Biol. Chem 132 171 1940
23 Tuchman L. R Suna H and Carr J J Elevation of serum acid phosphatase in Gaucher's disease J Mt Sinai Hosp, 23 227 1956
23a Crocker A. C., and Landing B H Phosphatase studies in Gaucher's disease. Metabolism 9 341 1960
24 Collier W A. A case of enlarged spleen in a child aged six Tr Path Soc London 46 148, 1895
25 Picou R. and Ramond R. Splénomegalie primitive épithélioma primitif de la rate Arch méd expér 8 168 1896
26 Petit J V and Schleicher E. M Atypical Gaucher's disease Am J Clin Path 13 260 1943
27 Pittaluga P G., and Goyanes J Contribution a l'étude de la cellule de Gaucher Arch mal coeur 26 65 1933
28 Block M., and Jacobson L. O The histogenesis and diagnosis of the osseous type of Gaucher's disease Acta haemat 1 165 1948
29 Risel W Über die grosszellige Splenomegalie (Typus Gaucher) und über das endotheliale Sarkom der Milz Beitr path Anat 46 241 1909
30 Mandlebaum F S and Downey H The histo-pathology and biology of Gaucher's disease (large-cell splenomegaly) Folia haemat., 20 139 1916
31 Kraus E. J Zur Kenntnis der Splenomegalie Gaucher insbesondere der Histogenese der grosszelligen Wucherung Ztschr angew Anat 7 186 1920
32 Oberling C La maladie de Gaucher Ann anat pathol et anat normale méd chir 3 353 1926
33 Bloom W Splenomegaly (type Gaucher) and lipoid histiocytosis Am J Path., 1 595 1925
34 Morrison R. W and Hack M H Histochemical studies in Gaucher's disease. Am J Path 25 497 1949
35 Kovacs K. Traut A. and Horvath F Cytochemistry of the Gaucher cell. Schweiz Ztschr allg Path 17 605 1954
36 Diezel P B Histochemische Untersuchungen an primaren Lipoidosen am aurotische Idiotie Gargoylismus Niemann Picksche Krankheit Gauchersche Krankheit mit besonderer Berucksichtigung des Zentralnervensystems Arch. path Anat 326 89 1954
37 Diezel P B Histochemical study of primary lipidoses in Cerebral Lipidoses edited by L. van Bogaert J N Cumings and A Lowenthal p 11 Blackwell Oxford 1957
38 Erf A E Studies of Gaucher cells by the supravital technique Am J M Sc., 195 144 1938
39 Cazal P Remarques sur la structure l'histogenèse et la signification de la cellule de Gaucher Sang 16 28 1944.
40 Kimmelstiel P and Laas E Morphologische Studien zur Frage des Lipoid antagonismus Beitr path Anat 93 417 1934
41 Pasternak, L. and Page I H. Das Schicksal intravenos injizierter Phosphatide Biochem Ztschr 252 254, 277
42 Dworacek E. and Pesta H Über einen spektralanalytischen Nachweis des Kerasins und experimentelle Cerebrosid speicherung im Sinne eines Morbus Gaucher Wien klin. Wchnschr 52 332 1939
43 Christianson O O Experimental lesions produced by cerebrosides Arch Path., 32 369 1941
44 Beumer H and Fasold H Versuche einer Cerebrosidspeicherung Ztschr ges. exper Med 90 661 1933
45 Motulsky A. G Casserd F Giblett E R. Broun, G O., and Finch, C A. Anemia and the spleen New England J Med 259 1164 1958
46 Medoff A S and Bayrd E D Gaucher's disease in 29 cases hematologic complications and effect of splenectomy Ann Int Med., 40 481 1954.
47 Kaplan M., Grumbach R. Fischgrund A. and Lunnel J Gaucher's disease

in a 6-year old child biological study of the following hemorrhagic syndrome Bull et mém Soc méd hôp Paris 69 169 1953
48 Kostich Yoksitia G A Case of Gaucher s disease Sang 23 586 1952
49 Morrison A N and Lane M Gaucher s disea e with ascites a case report with autopsy findings Ann Int Med 42 1321 1955
50 Edlin I Kepler W E Jr and Knabe G W Gaucher s disease Gastro enterology 28 1 0 1955
51 Pachman D J Chronic Gaucher s disease Am J Dis Child 56 218 1938
52 Goldfarb A R Atlas D H and Baberman P Electrophoretic studies in Gaucher s disease Am J Clin Path 20 963 1950
53 Gordon G L Osseous Gaucher s disease Am J Med 8 33 1950
54 Junghägen S Röntgenologische Skelettveranderungen bei Morbus Gaucher Acta radiol 5 506 1926
55 Davies F W T *Gaucher s disease J Bone & Joint Surg* 34 454 1952
56 Brill N E Primary splenomegaly Am J Med 121 377 1901
57 East T and Savin L H A case of Gaucher s disease with biopsy of typical pingueculae Brit J Ophth 24 611 1940
58 Bloem T F Groen J and Postma C Gaucher s disease Q tart J Med 5 517 1936
59 Mandlebaum H Barber L Lederer M Sobel A E and Kaye I A Gaucher s disease Ann Int Med 16 438 1942
60 Wechsler H F and Gustafson E Gaucher s disease New York J Med 40 133 1940
61 Marklen P Waitz R and Warter J Diagnosis of Gaucher s disease by splenic puncture Bull et mém Soc méd hôp Paris 49 36 1933 cited by Thannhauser (ref 9)
62 Case records of the Massachusetts General Hospital New England J Med 222 680 1940
63 Horsley J S Jr Baker J I and Apperly F L Gaucher s disea of late on et with kidney involvement and huge spleen Am J M Sc 190 511 1935
64 Kohne G Über Morbus Gaucher mit Hirnveranderungen Ztschr path Anat 102 512 1939
65 Aball A J and Kato K Gaucher s disea e in early infancy review of literature and report of case with neurological symptom J Pediat 13 364 1938
66 De Lange C Über die maligne Form der Gaucherschen Krankheit Acta paediat 27 34 1940
67 Campolo A Über die Pathologie der Gauscherschen Krankheit im fruhen Kindesalter (mit besonderer Berucksichtigung der neurologischen Form) Acta pediat 37 6 1949
68 Brain R Kerasin thesaurismosis Rev neurol 89 319 1953
69 Delré R Bertrand I Crumlach R and Bargeton C Malad e de Gaucher du nourrisson Arch franç pédiat 8 38 1951
70 Teilum C Die Gaucher'sche Krankheit mit der Beschreibung eines Falles der Veranderung n in der Hypophyse und im Hypothalamus zeigte Acta med s and nav 116 1 0 1944
71 Rodgers C C and Jackson B H Acute infantile Gaucher s disease Pediatrics 7 53 1951
72 Mislow K The geometry of sphingosine J Am Chem Soc 74 5155 1953
73 Marinetti G and Stotz E Studies on the structure of sphingomyelin IV Configuration of the double bond in sphingomyelin and related lipids and a study of their infrared spectra J Am Chem Soc 76 1347 1954
74 Carter H E and Fujino Y Biochemistry of the sphingolipides IX Configuration of the cerebrosides J Biol Chem 221 8 9 1956
75 Carter H E Sphingolipides in *Chemistry of Lipides as Related to Atherosclerosis* edited by I H Page p 8 Charles C Thomas Springfield Ill 1958
76 Carter H E Norris W P Glick F J Phillips C E and Harris R Biochemistry of the sphingolipides II Isolation of dihydrosphingosine from the cerebroside fraction of beef brain and spinal cord J Biol Chem 170 6 9 1947
77 Klenk E *Die Chemie der Cerebroside und Phosphatide* Springer Berlin 1930

78 Chibnall A C Piper S H and Williams E F XVIII The fatty acids of phrenosin and kerasin Biochem J 30 100 1936
79 Page I H *Chemistry of the Brain* Charles C Thomas Springfield Ill 1937
80 Thierfelder H Über das Cerebron Ztschr physiol Chem 43 21 1904
81 Levene P A On the cerebrosides of brain tissue J Biol Chem 15 364 1913
82 Thierfelder H Untersuchungen uber die Cerebroside des Gehirns Ztschr physiol Chem 85 35 1913
83 Rosenheim O XV The galactosides of the brain IV The constitution of phrenosin and kerasin Biochem J 10 142 1916
84 *Klenk E Über die Cerebroside des Gehirns Ztschr physiol Chem 166 268* 1927
85 Klenk E Über die Oxysäuren der Cerebroside des Gehirns Ztschr physiol Chem 174 214 1928
86 Klenk E Über ein neues Cerebrosid des Gehirn Ztschr physiol Chem 145 244 1925
87 Klenk E Beitrage zur Chemie der Lipoido en Ztschr physiol Chem 267 128 1940
88 Klenk E and Schumann E Über das Vorkommen einer n Hexaco en aure unter den Fettsauren der Gehirncerebroside Ztschr physiol Chem 272 1 1941
89 Klenk E and Leupold F Über eine vereinfachte Methode zur Darstellung von phosphorfreien Cerebrosiden und uber die als Spaltprodukte auftretenden Fettsauren Ztschr physiol Chem 281 208 1944
90 Chibnall A C Piper S H and Williams E F Further observations on phrenosinic acid Biochem J 55 707 1953
91 Klenk E and Faillard H Zur Kenntnis der Fettsauren der Gehirncerebroside Die Konstitution der ungesattigten Oxysauren Ztschr physiol Chem 292 268 1953
92 Skipski V P Arfin S M and Rapport M M Identification of 2 hydroxy stearic acid in spinal cord phrenosine by chromatographic separation of hydroxy fatty acids Arch Biochem 82 487 1959
93 Kishimoto Y and Radin N S Isolation and determination methods for brain cerebrosides hydroxy fatty acids and unsaturated and saturated fatty acid J Lipid Res 1 72 1959
94 Kishimoto Y and Radin N S Composition of cerebroside acids as a functi n of age J Lipid Res 1 79 1959
95 Thierfelder H Ueber die Identitat des Gehirnzuckers mit Galactose Ztschr physiol Chem 14 209 1890
96 Brown H T and Morris G H VIII Note on the identity of cerebrose and galactose J Chem Soc 57 57 1890
97 Klenk E and Rennkamp F Über die Ganglioside und Cerebroside der Rindermilz Ztschr physiol Chem 273, 253 1942
98 Brante G Studies on the lipids in morbus Gaucher I Qualitative and quantitative detemination of the hexose components in normal and Gaucher glycolipids Acta Soc med Upsalien 56 125 1951
99 Hsien Gieh S Faust C E and Williams H H Studies on cerebrosides Fed Proc 7 161 1948
100 Klenk E The pathological chemistry of the developing brain in *Biochemistry of the Developing Nervous System* edited by E Waelsch p 397 Academic Press Inc New York 1955
101 Montreuil J Boulanger P and Houcke E Chromatographie sur papier des constituants glucidique des cérébrosides d une rate de Gaucher Bull Soc chim biol 35 1125 1953
102 Carter H E and Greenwood F L Biochemistry of the sphingolipides VII Structure of the cerebrosides *J Biol Chem 199 283 1952*
103 Nakayama T Studies on the conjugated lipids I On the configuration of cerebrosides J Biochem (Japan) 37 309 1950
104 Hamasatu Y Chemi try and biology of lipids III On psychosine Tohoku J Exper Med 53 35 1950

105 Kiss J and Juresik I Investigations in the sphingo ine field Part XIV The structure of sphingoglycosides Acta chim Acad sci Hung 5 477 1954
106 Rosenberg A and Chargaff E A reinvestigation of the cerebroside deposited in Gaucher s disease J Biol Chem 233 1323 1958
107 Radin N S Lavin F B and Brown J R Determination of cerebroside J Biol Chem 217 789 1955
108 Edgar G W F Myelination studied by quantitative determination of myelin lipids Part I Acta anat 27 210 1956
109 Svennerholm L The quantitative estimation of cerebrosides in nervous tissue J Neurochem 1 32 1956
110 Weiss B The separation of sphingolipides by adsorption chromatography J Biol Chem 223 523 1956
111 Klenk E and Lauenstein K Über die zuckerhaltigen Lipoide der Formbestandtiele des menschlichen Blutes Ztschr physiol Chem 288 [illegible] 1951
112 Uzman L L and Rumley M K Changes in the composition of the developing mouse brain during early myelination J Neurochem 3 170 1958
113 Johnson A C McNabb A R and Rossiter R J Concentration of lipids in the brain of normal infants and adults Biochem J 44 494 1949
114 Johnson A C McNabb A R and Rossiter R J Lipids of normal brain Biochem J 43 573 1948
115 Brante G Studies on lipids in the nervous system with special reference to quantitative determination and topical distribution Acta physiol scandinav 18 Suppl 63 1 1949
116 Ottenstein B Schmidt G and Thannhauser S J Studies concerning the pathogenesis of Gaucher s disease Blood 3 1250 1948
117 Robins E Eydt K M and Smith D E Distribution of lipides in the cerebellar cortex and its subjacent white matter J Biol Chem 220 6 7 1956
118 Koch W and Koch M L Contributions to the chemical differentiation of the central nervous system III The chemical differentiation of the brain of the albino rat during growth J Biol Chem 15 423 1913
119 Folch J Composition of the brain in relation to maturation in *Biochemistry of the Developing Nervous System* edited by H Waelsch p 121 Academic Press Inc New York 1955
120 Bürger M Das Altern des Zentralnervensystems in *Experimental Research in Ageing* edited by F Verzar Berhauser Verlag Basal Experientia Suppl 4 p 101 1956
121 Edgar G W F Myelination studied by quantitative determination of myelin lipids Part II Acta anat 31 451 1957
122 Svennerholm E and Svennerholm L Determination of glucolipids in plasma Acta chem scandinav 10 1048 1956
123 Burt N S and Rossiter R J Lipids of rabbit blood cells Data for red cells and polymorphonuclear leucocytes Biochem J 46 569 1950
124 Meltzer H L Counter-current separation of brain lipides Fed Proc 14 100 1955
125 Burton R M Sodd M A and Brady R O Incorporation of galactose into galactolipids by brain tissue Fed Proc 16 161 [illegible]
126 Radin N S Martin F B and Brown J R Galactolipide metabolism J Biol Chem 224 499 1957
127 Burton R M Sodd M A and Brady R O The incorporation of galactose into galactolipides J Biol Chem 233 1053 1958
128 Moser H W and Karnovsky M L Studies on the biosynthesis of glycolipides and other lipides of the brain J Biol Chem 234 1990 1959
129 Moser H and Karnovsky M L Studies on the biosynthesis of cerebroside galactose Neurology 8 Suppl 1 81 1958
130 Mandelstam P and Burton R M Incorporation of glucose and galactose into glycolipids of rat brain tissue Fed Proc 18 80 [illegible]
131 Cleland W W and Kennedy E P Incorporation of C labeled galactose-1 P and uridine diphosphate galactose into brain lipides Fed Proc 17 [illegible] 1958
131a Burton R M and Brady R O Personal communication

132 Meyerhof O and Aeluzkoma N The rate of anaerobic glycolysis of various hexo es in mammalian ti ues Arch Biochem 12 405 1J1
133 Slein M W Cori G T and Cori C F A comparative study of hexokinase from yeast and animal tissues J Biol Chem 186 763 1950
134 Leloir L F Enzymic isomerization and related proces es Advances Enzymol 14 193 1953
135 Maxwell L S Kalckar H M and Burton R M Galacto-waldena e and the enzymic incorporation of galacto e 1 phosphate in mammalian tissues Biochim et biophys acta 18 444 1955
136 Kurahashi K cited by Burton et al (ref 12?)
137 Trams E G and Brady R O Studies on Gaucher s disea e Fed Proc 18 340 1959
138 Thannhauser S J and Fränkel E Über das Lignocerylsphingosin Zur Kenntnis des sogenannten Unverseifbaren der Saugetierleber Ztschr physiol Chem 203 183 1931
139 Tropp C and Wiedersheim V Untersuchungen über Lipoide tierischer Organe Über das Vorkommen des Lignoceryl sphingosins in der Rindermilz Ztschr physiol Chem 222 39 1933
140 Thannhauser S J and Reichel M Studies on animal lipids X The nature of cerebrosidase Its relation to the splitting of polydiaminopho phatide by polydiaminophosphatase J Biol Chem 113 311 1936
141 Fujino Y Studies on the conjugated lipids V On the enzymatic hydroly is of cerebron J Biochem (Japan) 40 251 1953
142 Johnson A C McNabb A R and Rossiter R J Chemical studies of peripheral nerve during Wallerian degeneration I Lipids Biochem J 45 500 1949
143 Rossiter R J in *Neurochemistry* edited by K A C Elliott I H Page and J H Quastel p 709 Charles C Thomas Springfield Ill 1955 cited by Radin et al (ref 126)
144 Mai H Chemische Untersuchung einer Gaucher Milz Ztschr Kinderh 55 12 1933
145 Lieb H Der Zucker im Cerebrosid der Milz bei der Gaucher Krankheit Ztschr physiol Chem 271 211 1941
146 Klenk E and Rennkamp F Der Zucker im Cerebrosid der Milz bei der Gaucher Krankheit Ztschr physiol Chem 272 280 1942
147 Iolonovski J Sur un glucosidolipide de la rate Compt rend acad sci Paris 215 443 1942
148 Danielson I S Hall C H and Everett M R Glucoside type of cerebro ide in spleen in Gaucher s disea e Proc Soc Exper Biol & Med 49 569 1942
149 Sacks M S and Andersch M A Isolation of a glucose containing cerebroside from the spleen in Gaucher s disease Am J M Sc 212 546 1946
150 Landolt R F Lollinger H V and Engster C H Helvet paediat acta 4 319 1948 cited by Brante (ref 98)
151 Halliday N Cerebrosides from spleen and brain from an adult with Gaucher s disease Proc Soc Exper Biol & Med 75 659 1950
152 Uzman L L The lipoprotein of Gaucher s disease A M A Arch Path 51 329 1951
153 Uzman L L Polycerebrosides in Gaucher s disea e I Isolation compo ition and physical properties A M A Arch Path 55 181 1953
154 Devor A W Carbohydrate residues in spleen cerebrosides Arch Biochem 50 217 1954
155 Iarke D V The occurrence of lactose in the spleen cerebrosides of a case of Gaucher s disease Biochem J 56 xv 1954
156 Woolf L I The sugar-containing lipids of Gaucher s disease Biochem J 56 xvi 1954
157 Devor A W Conger C and Gill I The use of resorcinol for identification and determination of monosaccharide groups a report on a Gaucher spleen cerebroside Arch Biochem 73 20 1958
158 Tuchman I R Goldstein G and Clyman M Studies on the nature of the

increased serum acid phosphatase in Gaucher s disease Am J Med 27 359 1959
159 Abul F Adl M A M and King L J Properties of the acid phosphatases of erythrocytes and human prostate gland Biochem J 45 51 1949
160 Hastrup B and Videbaek A Acid phosphatase in Niemann Pick disease and a therapeutic experiment with cortisone Acta med scandinav 149 287 1954
161 Kessel A W L and Signy A G Acid hyperphosphatasia in three families with osteogenesis imperfecta Lancet 2 1317 1956
162 Tuchman L R Personal communication
163 Decker B and McWhorter C A Gaucher s disease and pregnancy Ann Int Med 44 1219 1956
164 Bromberg Y M Touff R and Diengott D Pregnancy and Gaucher s disease Brit M J 4839 761 1953
165 Groen J The hereditary mechanism of Gaucher s disease Blood 3 1 38 1948
166 Brill N E Mandlebaum F S and Libman S Primary splenomegaly Gaucher type Am J M Sc 129 491 1905
167 Spackman W C and Mackie F P Gaucher s type of splenomegaly in a Mahratta village with a case treated by splenectomy Indian M Gaz 40 69 19 5 cited by Herndon and Bender (ref 172)
168 Stransky E and Dauis-Iawas D T Heredity in the infantile type of Gaucher s disease report of a case Am J Dis Child 78 691 1949
169 *Stransky L and Conchu T L Heredity in the infantile type of Gaucher s* disease Ann paediat 177 319 1955
170 Medal L S Trevine A and Palizza G Gaucher s disease Rev invest clin 6 417 1954
171 Reiss O and Kato K Gaucher s disease Am J Dis Child 43 365 1932
172 Herndon C N and Bender J R Gaucher s disease cases in 5 related Negro sibships Am J Human Genet 2 49 1950
173 Hsia D Y Naylor J and Bigler J A Gaucher s disease Report of two cases in father and son and review of the literature New England J Med 261 164 1959
174 Marinetti G V Ford T and Stotz L The structure of cerebrosides in Gaucher s disease J Lipid Res 1 203 1960

Part Five

# Disorders of Steroid Metabolism

# Chapter 20

# The Adrenogenital Syndrome

---

*Gordon M. Tomkins and Joseph S. McGuire, Jr.*

The adrenogenital syndrome is usually manifested by pseudohermaphroditism of females and premature virilization of males and may occur spontaneously or may result from various endocrine disorders such as tumors of the adrenal glands or gonad. The most frequent cause is congenital adrenal hyperplasia. Virilization of females and premature maturation of males are arresting clinical phenomena, and individuals with the adrenogenital syndrome have attracted considerable medical interest.

Although many patients in earlier reports may have had the adrenogenital syndrome, the first convincing descriptions were by Otto in 1816, Crecchio in 1866, and Marchand in 1891 (cited by Bulloch [1]). In 1872 Ogston [2] described three female pseudohermaphrodites with bilateral adrenal hyperplasia, and in 1910 Apert [3] published an important review in which the effects of adrenal hyperplasia were classified according to age of onset, a classification that is still of use. Gallais can be credited with naming the syndrome *le syndrome génitosurrénal* in 1912 [4], and Glynn in the same year [5] reported several patients with the adrenogenital syndrome, four of whom were boys.

## CLINICAL OBSERVATIONS

The syndrome, of which there are several distinguishable forms, is caused by heritable defects in the biosynthesis of steroids by the adrenal gland. These defects result directly and indirectly in the overproduction of certain steroids, some of which are androgenic. The cardinal sign of this syndrome is virilization, but the clinical manifestations depend upon the genetic sex, age of onset, and type and degree of the metabolic defect [6–12].

The patients can be conveniently divided into four groups according

to etiology and clinical expression (1) virilization (the major form) (2) virilization with adrenocortical insufficiency, (3) virilization with hypertension (4) virilization with adrenocortical insufficiency and hypertension

The age of onset of virilization is most commonly prenatal but is probably not before the fifth month of embryonic development [13] Females exhibit a variety of malformations of the genitalia and sex determination is sometimes difficult because affected females resemble male pseudohermaphrodites Chromosomal sex can be determined by skin biopsy [14] or by examination of oral mucosal smears [15 16] The clitoris is enlarged and may enclose the urethra for a variable distance Rarely the urethra may be completely penile [17] but more commonly there is a groove on the ventral surface of the clitoris which joins the urogenital sinus The labia minora are small or absent The labia majora are hypertrophied rugose pigmented and partially fused posteriorly (Fig 20-1) Virilization likewise takes place in males but is often overlooked for variable periods because of its isosexual nature Both sexes show accelerated somatic maturation Bone age and growth are advanced The epiphyses close prematurely and a growth deficit results Pubic hair may appear in the second year acne is common and there is pigmentation of the scrotum or labia and nipples In the male the testes remain small and spermatogenesis does not occur [18 19] Females usually have amenorrhea accompanying progressive virilization marked by male muscular development male hair distribution growth of beard and often temporal or frontal baldness Breast development is slight and the voice is lowered in pitch

When the adrenogenital syndrome is not present at birth but occurs in the prepubertal period it is almost always due to adenoma or adenocarcinoma of the adrenal cortex Progressive virilization takes place as in the congenital form and in the female is superimposed on normally developed genitalia

When the syndrome occurs after puberty in females menstruation becomes less regular and then ceases Acne and hirsutism appear the genitalia atrophy the clitoris enlarges and the breasts regress The voice deepens and the sex drive is often accentuated The postpubertal onset of the syndrome has also been described in an adult male [20]

Less severe conditions which may be variants of the adrenogenital syndrome also occur in women These individuals are hirsute and may have enlargement of the clitoris as well as menstrual and ovulatory disturbances [21–24]

## ADRENOCORTICAL INSUFFICIENCY

In approximately half the patients with congenital adrenal hyperplasia there are in addition to virilism adrenal insufficiency and associated

disturbances of salt and water metabolism as described first by Butler et al [25] and later by others [19 26–32] The e abnormalities characterized by urinary loss of sodium and chloride appear in the first week in half of those ultimately affected and by 7 weeks of age in almost all [33] The infants are apathetic Vomiting is the rule diarrhea is common and dehydration almost invariably occurs The life-threatening complication is circulatory collapse comparable to that in Addisonian crisis While the diagnosis suggests itself in females because of the associated genital malformations it is often difficult in males At birth most of the males have genitalia of normal size but all show signs of virilization by 2 years of age The fact that death occurred within the first 13 months in a large number of these children who did not receive specific treatment emphasizes the importance of prompt diagnosis in this group

### HYPERTENSION

Another group of patients [34 35] with the adrenogenital syndrome has in addition to progressive virilization hypertension which can be severe enough to produce cardiomegaly and heart failure Changes in retinal blood vessels consistent with hypertension have been een This hypertension reflects a defect in steroid metabolism different from that present in the other groups

### HYPERTENSION AND ADRENOCORTICAL INSUFFICIENCY

In the past few years three adrenogenital infants who have a hitherto unde cribed clinical form of the di ease have been studied by Bartter and Fronove [36] (Fig 20-1) They exhibited in addition to hypertension signs of adrenal insufficiency characterized by ensitivity to salt deprivation Two of the patients are female p eudohermaphrodites and the third is a brother of one of them

## CHOLESTEROL BIOSYNTHESIS

Prior to a discussion of the altered teroid synthesis as ociated with congenital adrenal hyperpla ia the normal pathways of steroid biosyn the is will be de cribed Chole terol is the mo t important if not indeed the sole precursor of the steroid hormones Before a discu sion of adrenal corticoid bio ynthe is can be undertaken attention mu t be devoted to the biochemical origins of this key metabolite

Because of the relatively large ize and complexity of chole terol (the structure was e tabli hed early in 1932 [37]) the problem of its bio yn the is was very difficult In its solution a vital role was played by the organic chemists who provided remarkably accurate prediction about routes and mechanisms in the complicated series of reactions leading to the sterols but it was only through pain taking carbon by-carbon de-

gradations of isotopically labeled cholesterol and its synthetic intermediates [38–41] that the presently accepted path of biosynthesis was verified



Fig 20-1 Female pseudohermaphrodite 14 months old with virilizing congenital adrenal hyperplasia thought at birth to be a male with hypospadias At 1 days because of cyanosis labored breathing and a cardiac arrhythmia she was admitted to a hospital where the diagnosis of congenital adrenal hyperplasia with adrenal insufficiency was made It is of particular interest that she not only had adrenocortical insufficiency with associated electrolyte disturbances but also was hypertensive with systolic blood pressures of 150 to 180 mm Hg and occasionally higher Without steroid therapy and added dietary salt hyperkalemia and hyponatremia appeared rapidly The elevated blood pressure and adrenal insufficiency are now corrected by the oral administration of 12 5 mg cortisol per day and added salt in her diet Several months after the photograph a clitoridectomy was performed with a satisfactory cosmetic result The patient is under the care of P Pronove and F C Bartter of the *National Institutes of Health (Permission of P Pronove and F C Bartter)*

## IN VIVO SYNTHESIS OF CHOLESTEROL FROM ACETATE

For the sake of clarity the biosynthetic reactions will be described as they occur sequentially rather than as they were unraveled historically First of course it was necessary to establish that mammalian organisms can in fact synthesize cholesterol Balance studies [42] demonstrated this since the total body cholesterol of the experimental animals remained constant even when they were placed on a cholesterol free diet The historic isotope experiments of Rittenberg and Schoenheimer [43] confirmed this view and in addition suggested that the sterol is made from small molecules rather than large preformed precursors Experiments conducted on ergosterol[1] formation in microorganisms [44] suggested that the precursor might be acetate Another approach made use of a *Neurospora* mutant which requires acetate for growth [45] and led to the same conclusion When the mold was grown on glucose in the presence of labeled acetate the isotope content of the sterol isolated from the cul

[1] This plant sterol which differs from cholesterol only by an additional methyl group and double bond in the side chain is sufficiently close structurally so that studies on its biogenesis have been of great help in understanding cholesterol biosynthesis

ture was the same as that of the isotopic precursor. This finding indicated that at least in this organism acetate is the sole sterol precursor. Many other experiments with mammalian organs, principally liver [46–48], have confirmed these earlier findings. More recently the stepwise degradation of cholesterol biosynthesized from either methyl- or carboxyl-labeled acetate-$C^{14}$ has firmly established what could only be inferred from the previous experiments, namely, that each carbon in the sterol molecule arises from either the carboxyl or methyl carbon of acetate [38–41, 49].

Especially relevant to present considerations is the work of Srere et al [50] who showed that the adrenal gland also can convert acetate to cholesterol. Later work by these investigators [51] has demonstrated that in fact almost all mammalian tissues can carry out this biosynthesis.

## ACETATE ACTIVATION

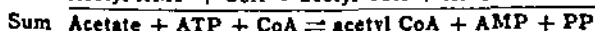
The extensive investigations of Lipmann and Lynen [see 52] and their respective collaborators have established that the free acetate ion ($CH_3COO^-$) does not itself participate in biosynthetic reactions but that it must first be metabolically "activated" by condensation with the pantothenic acid–containing coenzyme A to form a thiol ester, acetyl coenzyme A. The formation of acetyl CoA has been shown by Berg [53, 54] to occur in two steps: first, a reaction of acetate with ATP to form acetyl adenylate (acetyl AMP), followed by transfer of the acetyl moiety to coenzyme as follows:



$$\text{Acetate} + \text{ATP} \rightleftharpoons \text{acetyl AMP} + \text{pyrophosphate (PP)} \qquad (1)$$



$$\text{Acetyl AMP} + \text{CoA} \rightleftharpoons \text{acetyl CoA} + \text{AMP} \qquad (2)$$

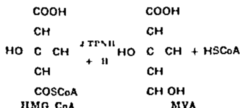


$$\text{Sum:}\quad \text{Acetate} + \text{ATP} + \text{CoA} \rightleftharpoons \text{acetyl CoA} + \text{AMP} + \text{PP}$$

## FORMATION OF MEVALONIC ACID

The earliest reaction of acetyl CoA in sterol formation is the condensation of two molecules to form acetoacetyl CoA, which in turn reacts with a third acetyl CoA to yield the CoA derivative of β-hydroxy-β-methylglutaric acid (HMG CoA) [55, 56] (Fig. 20-2).

Recently [57] the further metabolism of HMG CoA has been shown to involve its reduction and deacylation to produce mevalonic acid (MVA) as follows:



This reaction is noteworthy in several respects It proceeds without the accumulation of an aldehyde intermediate mevaldic acid Wright [58] and Lynen [59] have shown however that the latter can be reduced to MVA when introduced into the appropriate system

In addition the HMG MVA conversion appears to be the rate limiting step in sterol biogenesis which means that physiologic influences affecting the rate of cholesterol synthesis [60] probably act on this reaction

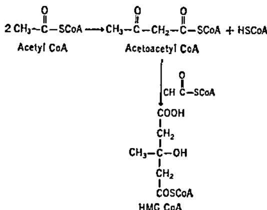



Fig 20-2 Biosynthesis of HMG CoA

## FORMATION OF SQUALENE

That MVA is an efficient cholesterol precursor was a finding of major importance since at the time it was the only definite link between acetate and the 30-carbon intermediate squalene MVA originally came to light as the acetate-replacing factor of *Lactobacillus casei* subsequently it was tested [61] as a sterol antecedant and found to be very effective Further work in which squalene [62 63] and cholesterol [64] biosynthesized from MVA $C^{14}$ were degraded has indicated that following decarboxylation the five remaining carbons of MVA are converted directly to the sterol The intermediate reactions are presently the subject of intensive investigation Enzyme studies [65] have shown that mevalonic acid is phosphorylated to phosphomevalonic acid (MVAP) by ATP in the presence of a yeast enzyme mevalonic kinase and that this phosphorylation is a prerequisite to the conversion of MVA to sterol More recent evidence [66] suggests that a further phosphorylation of MVAP also at the expense of ATP yields MVA pyrophosphate (MVAPP) which can lose water and $CO_2$ to form isopentenyl pyrophosphate The latter isomerizes according to a preliminary report [67] to form $\gamma\gamma$ dimethylallyl pyrophosphate (Fig 20 3)

On the basis of indirect evidence Rilling et al [68] had concluded that the hydrocarbon farnesene ($C_{15}H_{25}$) was intermediate between MVA

and squalene More recently the Lynen group [69] has shown that the intermediates between isopentenol PP and squalene are geranyl PP and farnesyl PP and that 2 moles of farnesyl PP can conden e in a reductive

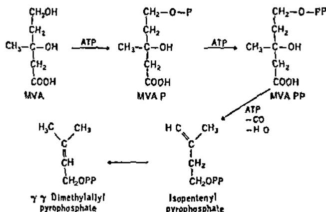



Fig 20-3 Conversion of MVA to γ γ-dimethylallyl pyropho phate

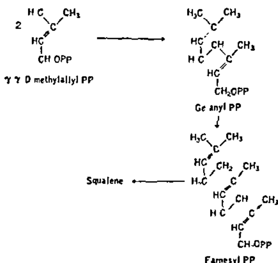



Fig 20-4 Formation of squalene from γ γ-dimethylallyl pyrophosphate

reaction to become squalene (Fig 20-4) They have also suggested a general mechanism for the bio ynthesis of other terpene derivatives via condensation of isopentenyl pyropho phate based on the bond energy of pyropho phoric acid esters and the tendency of these un aturated compounds to form carbonium ion intermediates

## CYCLIZATION OF SQUALENE

It is interesting that as early as 1926 it was suggested that squalene was a cholesterol precursor [70 71] because when it was fed to rats their liver and blood cholesterol concentrations increased Shortly thereafter Sir Robert Robinson [72] suggested a plausible mechanism by which squalene could cyclize to produce a sterol Nearly 30 years later, this suggestion and a much newer alternate hypothesis advanced by Woodward and Bloch [73] were tested, and the former was rejected The role of squalene as a sterol precursor however, has been quite definitely established

Closure of the squalene molecule which results in the first sterol intermediate, lanosterol [74] (Fig 20 5) is one of the most complicated

O TPNH HO

Squalene Lanosterol

Fig 20-5 The cyclization of squalene Arrows indicate movement of electron pairs

apparently single step biochemical reactions known An ingenious mechanism for it has been proposed by Ruzicka [75] in which a cationic oxygen atom attacks what will become carbon number 3 of the steroid nucleus There follows a series of electron displacements carbonium ion intermediates and 1 2 methyl shifts and a final stabilization of the structure by ejection of a proton These brilliant speculations have not yet been decisively established but they are rendered probable by the findings that the entire squalene-lanosterol conversion proceeds without the uptake of hydrogen from the aqueous medium and that the oxygen introduced at carbon 3 is derived from atmospheric oxygen [76]

## CONVERSION TO CHOLESTEROL

The conversion of lanosterol ($C_{30}$) to cholesterol ($C_{27}$) (Fig 20-6) requires the removal of three extra methyl groups which are eliminated as $CO_2$ [77] the reduction of two double bonds one at position 8 and the other in the side chain and finally unsaturation at carbon 5

The available evidence [78–80] suggests that first the methyl group at position 14 and then the *gem* dimethyl groups at 4 are removed to produce zymosterol There follows the formation of desmosterol in which the double bond, previously at 8 has been shifted to position 5 The introduction of the latter unsaturations may proceed by hydroxylation at either 5 or 6 followed by removal of water Finally cholesterol is produced by reduction of 24–25 unsaturation

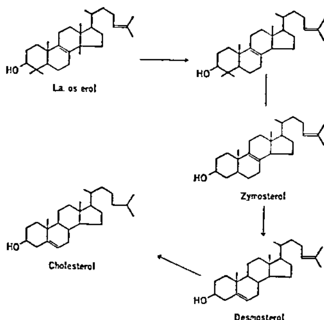



Fig 20-6 Biosynthesis of cholesterol from lanosterol

## ADRENOCORTICAL STEROID BIOSYNTHESIS FROM CHOLESTEROL

Cholesterol is a precursor of the adrenal steroids under many experimental circumstances As with cholesterol biogenesis the insight of the organic chemists preceded experimental verification of the synthetic routes The first demonstration that cholesterol is a steroid hormone precursor was by Bloch [81] who showed that deuterium labeled cholesterol is converted to labeled pregnanediol The latter was isolated from the urine of a pregnant woman The conversion of cholesterol to the corticoids has also been found both in vivo [82] and in vitro [83]

### PREGNENOLONE FORMATION

The initial attack on the cholesterol molecule results in cleavage of the side chain to yield i ocaproic acid and pregnenolone [84 85] a 21 carbon steroid which retains the 3β hydroxyl group of cholesterol and its 5-6 unsaturation (Fig 20-7) The detailed mechanism of conversion is not known There is evidence to suggest that 20-hydroxycholesterol may be an intermediate Enzyme studies have shown that the cleavage reaction is in some way dependent on DPN and ATP but the necessity for these coenzymes does not at the moment help in understanding the reaction mechanism

CYCLIZATION OF SQUALENE

It is interesting that as early as 1926 it was suggested that squalene was a cholesterol precursor [70 71] because when it was fed to rats their liver and blood cholesterol concentrations increased Shortly thereafter Sir Robert Robinson [72] suggested a plausible mechanism by which squalene could cyclize to produce a sterol Nearly 30 years later this suggestion and a much newer alternate hypothesis advanced by Woodward and Bloch [73] were tested and the former was rejected The role of squalene as a sterol precursor however, has been quite definitely established

Closure of the squalene molecule which results in the first sterol intermediate lanosterol [74] (Fig 20 5), is one of the most complicated

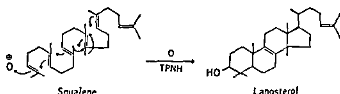



Fig 20-5 The cyclization of squalene Arrows indicate movement of electron pairs

apparently single-step biochemical reactions known An ingenious mechanism for it has been proposed by Ruzicka [75] in which a cationic oxygen atom attacks what will become carbon number 3 of the steroid nucleus There follows a series of electron displacements carbonium ion intermediates and 1 2 methyl shifts and a final stabilization of the structure by ejection of a proton These brilliant speculations have not yet been decisively established but they are rendered probable by the findings that the entire squalene-lanosterol conversion proceeds without the uptake of hydrogen from the aqueous medium and that the oxygen introduced at carbon 3 is derived from atmospheric oxygen [76]

CONVERSION TO CHOLESTEROL

The conversion of lanosterol ($C_{30}$) to cholesterol ($C_{27}$) (Fig 20-6) requires the removal of three extra methyl groups which are eliminated as $CO_2$ [77] the reduction of two double bonds one at position 8 and the other in the side chain and finally unsaturation at carbon 5

The available evidence [78–80] suggests that first the methyl group at position 14 and then the *gem* dimethyl groups at 4 are removed to produce zymosterol There follows the formation of desmosterol in which the double bond previously at 8 has been shifted to position 5 The introduction of the latter unsaturations may proceed by hydroxylation at either 5 or 6 followed by removal of water Finally, cholesterol is produced by reduction of 24–25 unsaturation

ensus recently summarized by Hechter [88] seems to be that pro gesterone is initially hydroxylated at 17 then at 21 and finally at 11 The mechanism of these hydroxylations is not well understood (see below) The enzyme system for 11 hydroxylation is found in the mito chondria [89] at least in bovine adrenal glands while the 17- and 21 hydroxylases are associated with the microsomal fraction [90 91] Each reaction requires the participation of TPNH

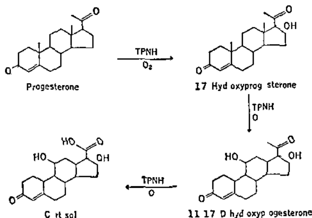



Fig 20-9 Biosynthesis of cortisol from progesterone

ALDOSTERONE FORMATION

Although not of principal concern here it is interesting to note that cholesterol can act as a precursor of the salt retaining hormone aldo sterone in cell free preparations of mammalian adrenal glands (reported in [88]) In addition to those enzymes mentioned earlier the only enzy matic machinery required for such a reaction is an 18-hydroxylase and a dehydrogenase capable of oxidizing the 18-hydroxyl group to an aldehyde and possibly several enzymes for the formation of the hemiacetals As with cortisol biosynthesis there is current disagreement about the order of the required hydroxylations when progesterone is the starting material The available evidence does not indicate a unique biosynthetic pathway from a particular $C_{21}$ aldosterone precursor but only shows that aldo sterone formation is possible from corticosterone cortexone (11-deoxy corticosterone) or progesterone

## BIOSYNTHESIS OF ADRENAL ANDROGENS

The preceding sections have shown how cholesterol is converted into adrenocortical hormones Perhaps the most remarkable feature of this pathway is that a single reaction hydroxylation occurs over and over

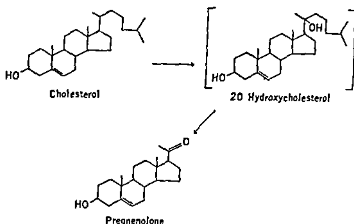



Fig 20-7 Pregnenolone formation from cholesterol

OXIDATION TO PROGESTERONE

The next step toward the corticoids is the transformation of preg nenolone into progesterone (Fig 20 8) Although the oxidation was initially assumed to be catalyzed by a single enzyme a $3\beta$ hydroxy steroid dehydrogenase [86] the process consists of two distinct chemical changes

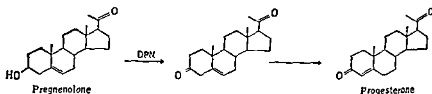



Fig 20-8 Conversion of pregnenolone to progesterone

in pregnenolone (1) oxidation of the hydroxyl group at 3 to form a ketone (2) isomerization from a 5 to a 4 unsaturated compound It seems much more likely therefore as suggested by Wang and Talalay [87] that the oxidation of the 3 hydroxyl group is catalyzed by one enzyme and the irreversible migration of the double bond is catalyzed by a second, a steroid isomerase

HYDROXYLATION OF PROGESTERONE

Progesterone when compared with the desired end product cortisol (Fig 20-9) lacks three hydroxyl groups $11\beta$ $17\alpha$ and 21 The order in which these are affixed to progesterone is debatable and it is not clear at present whether there is any required sequence of hydroxylation A reasonable amount of evidence suggests that proge terone can be hy droxylated initially in any of the three required positions but the con

## ESTROGEN BIOSYNTHESIS

The biosynthesis of the steroids has been pursued from their origins in acetic acid through the 30 carbon hydrocarbon squalene to cholesterol the corticoids and finally the $C_{19}$ androgenic hormones One further synthetic pathway remains to be examined estrogen biosynthesis Although only small quantities of estrogens have been isolated from bovine adrenal gland and the amount released to the circulation from the adrenal gland in individuals of either sex is probably insignificant an account will be given of the progress in aromatic steroid biogenesis since the role of the estrogens in adrenal physiology may be important

A d ostenedione — TPNH, O → Estrone

Fig 20-11 Formation of estrone from androstenedione

Only quite recently experimental evidence has indicated [95] that cholesterol is a precursor of the estrogens Before this was established the aromatic nature of the estrogens led some investigators [96] to postulate a cholesterol independent pathway possibly involving the aromatic amino acids as precursors of the estrogenic A ring New data permit a unified scheme in which cholesterol is transformed by way of the androgens to the 18 carbon female hormones The critical reactions are loss of the angular methyl group (C 19) attached to C-10 of the steroid nucleus and aromatization of the A ring Ryan [97] has recently found that microsomes prepared from human placenta can catalyze these reactions so that 4 androstene 3 17 dione is converted to estrone (Fig 20-11) The sequence of steps and the detailed mechanism are unknown although it appears that removal of the angular methyl group is somehow coupled to the aromatization since the 19 nor steroids (i e the compound lacking the angular methyl) will not react The requirements for the conversion i e TPNH and oxygen strongly suggest that at least one hydroxylation reaction is involved Although these reactions have not been studied in the adrenal gland the presence of estrogens in this tissue probably means that the same enzymatic process can also occur

## STEROID HORMONE BIOSYNTHESIS NOT INVOLVING CHOLESTEROL

Although cholesterol is an important precursor of the steroid hormones the question remains whether it is an obligatory precursor or whether there is another route not involving cholesterol

again as the molecule is altered sequentially It should come as no surprise then that this same mechanism is important in androgen biosynthesis

Androgen production from cholesterol [88] and progesterone [92] has been demonstrated in adrenal tissue Although these conversions have not been studied so extensively as in the testis it may be assumed that the reactions do not differ significantly in the two organs In testis the key finding has been that 4 androstenedione can be formed by cleavage of the 2 carbon side chain of 17α hydroxyprogesterone [93 94] (Fig 20 10) The synthetic sequence leading from progesterone to androgens

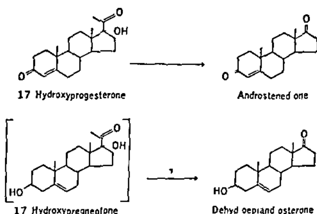



Fig 20-10 Conversion of a $C_{21}$ to a $C_{19}$ steroid

appears therefore to be first 17 hydroxylation of progesterone (which has already been discussed) followed by loss of the side chain by what appears to be a hydroxylation The two carbons eventually appear as free acetic acid

An unsettled problem concerns the origin of dehydroepiandrosterone (3β hydroxy androst 5-ene 17 one) Because it retains two of the characteristic features of cholesterol (a double bond at 5 and the 3β hydroxyl group) it is apparently not derived from progesterone or 17 hydroxy progesterone Two plausible schemes have been advanced to explain its presence The first which involves a direct synthesis from cholesterol with removal of the 8-carbon side chain is not supported by direct evidence The second which requires less revision of established pathways proposes by analogy with the 17 hydroxyprogesterone to androstenedione conversion that dehydroepiandrosterone arises from 17 hydroxypregnenolone Unfortunately in his study of the latter Lynn [94] did not test 17 hydroxypregnenolone as a substrate although the reaction appears probable

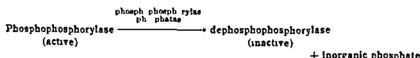
There are small differences two amino acids between the species Secondary structure (e g $\alpha$ helical configuration) appears to be lacking since no evidence of hydrogen bonded interaction has been obtained from deuterium exchange experiments [*109*]

A remarkable coincidence has appeared as a result of work on the ACTH structure The melanocyte-stimulating hormone (MSH) the structure of which has recently been determined [*110 111*] shares a sequence of seven amino acids with ACTH Thus it appears that the two peptides physiologically so different must have both genetic and functional characteristics in common Of incidental interest is that the common sequence explains the inherent MSH activity of purified ACTH [*112*] The structure of ACTH has not been confirmed by synthesis but work has already been carried to the point where peptides with MSH activity have been prepared [*113–115*]

Before discussing ACTH action it is necessary to examine some aspects of carbohydrate metabolism The investigations of Sutherland and his collaborators [*116–118*] on phosphorylase have been illuminating This protein which catalyzes the reversible phosphorolysis of glycogen




exists in both an active and an inactive form The active molecule in which one of the serine residues is phosphorylated [*119*] is termed phosphophosphorylase and can be inactivated by a second enzyme phosphophosphorylase phosphatase [*117*] which hydrolyzes the phosphate from the phosphoserine




Active phosphorylase can be regenerated by phosphorylation of the serine residue with ATP in the presence of still a third enzyme dephosphophosphorylase kinase [*116*]




This remarkable cycle has important physiologic consequences as concurrent work in Sutherland s laboratory has shown [*120*] The hormones epinephrine and glucagon which facilitate glycogen breakdown do so by *stimulating the phosphorylation of inactive phosphorylase* through the kinase reaction thereby activating inactive dephosphophosphorylase Stimulation of the kinase is affected in some way by a

The evidence for an alternate pathway in which cholesterol is not an intermediate is derived from isotope experiments using precursors of both cholesterol and the hormones, e.g., acetate. Under some circumstances, after incubation of adrenal tissue with such a labeled compound, the cholesterol isolated at the end of the experiment has had a lower specific activity than the hormones which supposedly were derived from cholesterol. Furthermore, Brady [98], using hog testis slices, obtained evidence that androgen biosynthesis from acetate in vitro could be stimulated by adding follicle-stimulating hormone (FSH) to the test system. Under these conditions there was no increase of acetate incorporation into cholesterol. Heard et al. [99] have reported that acetate-C is converted to various corticoids by hog adrenal homogenates and that the adrenal cholesterol does not become labeled at all. Unfortunately, these experiments cannot be interpreted unequivocally to mean that cholesterol is not a precursor. Since cholesterol is not uniformly distributed throughout the cell [100], it is possible that acetate might pass through a very small but metabolically active cholesterol pool on its way to the hormones without labeling the total intracellular cholesterol to a significant degree. Brady's experiments could be explained if the rate-limiting FSH-controlled reaction is between cholesterol and the androgens. An increase in the rate of such a process could then increase the isotope incorporation into the hormone without affecting the labeling of the cholesterol. In a recent review [88] Hechter suggested that a smaller isoprenoid intermediate than squalene, for example a 20-carbon hydrocarbon, could give rise to $C_{19}$ steroids and other steroids having fewer carbon atoms than cholesterol. Unfortunately, there is too little evidence to decide on the merits of such a hypothesis at the moment.

## CONTROL OF ADRENOCORTICAL FUNCTION

For a number of years the feed back mechanism involving the anterior pituitary and the adrenal cortex has been under intensive study by physiologists and clinicians. It has been known for some time [101–103] that the pituitary elaborates an adrenocorticotropic hormone (ACTH) which stimulates the adrenal cortex to increase its output of cortical steroids [104]. There is an accompanying fall in adrenal cholesterol and ascorbic acid [105]. 11-Hydroxylated cortical steroids inhibit the output of ACTH by the pituitary. Thus a homeostatic mechanism is present which serves to maintain a constant output of the cortical hormones.

As a result of many brilliant recent studies of the pituitary-adrenal relationship, a molecular basis for understanding the physiologic events is now available. One of the most striking advances has been the elucidation of the complete amino acid sequence of ACTH from both pig [106, 107] and sheep [108]. Both are polypeptides composed of 39 amino acids

A po sible explanation for the discrepancy may be that after the 18 hydroxylation required for aldosterone bio ynthe is there must be an oxidation to form the 18 aldehyde If this oxidative step were in fact very low it might limit the over all rate of aldosterone synthesis and therefore no matter how much the preceding hydroxylation reactions were enhanced by ACTH the rate of aldosterone formation would go no faster than the rate of the oxidation reaction Aldosterone production would therefore not be appreciably affected by ACTH but would be influenced by any stimulus which controls the rate of aldehyde formation at C-18

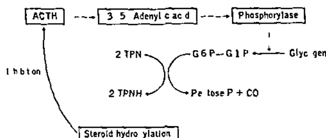



Fig 20-13 Mechanism of action of ACTH

Another unresolved feature of ACTH action is that it stimulates not only cortical secretion but also growth In addition to promoting TPNH production ACTH would provide pentose phosphate since that is a product of the oxidative metabolism of glucose 6 phosphate It might be that the concentration of pentose phosphate a nucleic acid con stituent controls the rate of growth of the adrenal gland In addition it is quite likely that once the primary action of ACTH is manifest secondary enzyme changes could occur as a re ult of enzyme induction for example which might result in an increase of adrenal size

The other limb of the feed back mechanism the means by which the steroids regulate pituitary ACTH output is a complete mystery It appears that pituitary suppressing activity of adrenal steroids cor responds to their glucocorticoid activity [*127*]

## THE MECHANISM OF STEROID HYDROXYLATION

The addition of hydroxyl groups to the steroids is of the utmost im portance in steroid metabolism Steroid hydroxylation was first en countered in the squalene oxidocycla e reaction where it was postu lated that a positive oxygen ion attacks squalene and initiates the dramatic ring closure in the formation of lanosterol This oxygen remains with the nucleus to become the 3β hydroxyl-oxygen of cholesterol In the conversion of lanosterol to cholesterol the three surplus methyl

cyclic nucleotide, 3',5' adenylic acid (Fig 20 12) This synthesis, from ATP in intracellular liver particles [121, 122] is stimulated by epinephrine and glucagon

Although these events have been shown to occur in liver, Haynes [123, 124] has obtained evidence that the situation in the adrenal gland is closely related Here ACTH (but not glucagon or epinephrine) stimulates the synthesis of the nucleotide On the other hand ACTH cannot stimulate synthesis of this nucleotide in liver At any rate once the 3' 5' adenylic acid is formed, its apparent function is stimulation of adrenal phosphorylase by the same mechanism as that outlined for the liver

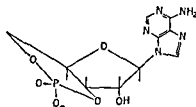


Fig 20-12 3' 5' Cyclic adenylic acid

The relation between the activation of phosphorylase by ACTH and increased cortical steroid biosynthesis has been explained by Haynes [123] He argues that since steroid hydroxylation reactions are quantitatively the most important steps in corticoid biogenesis and since TPNH is required for each hydroxylation the rate of TPNH formation in the adrenal gland could control the rate of steroid hydroxylation and, therefore steroid genesis

How can increase in phosphorylase activity produce a higher TPNH concentration? Under physiologic conditions phosphorylase catalyzes the breakdown of glycogen to yield glucose 1 phosphate which is isomerized to glucose 6 phosphate The latter can traverse one of two metabolic routes the Embden Meyerhof pathway, which results in the formation of pyruvate and DPNH or the oxidative pathway which yields 1 mole of pentose and 2 moles of TPNH per mole of glucose 6 phosphate oxidized It has been demonstrated [125] that the oxidative pathway in adrenal tissue is very active and consequently a large proportion of the glucose 6 phosphate derived from glycogen phosphorolysis will provide TPNH The reduced coenzyme can then be used for steroid hydroxylation [126] and therefore for corticoid androgen and incidentally estrogen biosynthesis This scheme is summarized in Fig 20 13

This sequence of coupled reactions is truly extraordinary The site of ACTH action is so many chemical steps removed from the enzymes directly concerned with production of steroids that a high degree of molecular organization is necessary for this control mechanism to be effective

Satisfying as the over all hypothesis may be several unanswered questions remain The first pertains to the lack of appreciable effect of ACTH on the formation of aldosterone This is surprising since the synthesis of aldosterone which is highly hydroxylated should be affected

react with oxygen to form a metal oxygen complex capable of hydroxylating the substrate

The role of TPNH is likewise not understood The important studies reported by Kaufman [139] on the conversion of phenylalanine to tyrosine have shown that TPNH is used to reduce a pteridine the function of which is still mysterious Other reports of additional requirements in steroid hydroxylation [140] may indicate that there too TPNH is only a secondary reducing agent Tchen and Bloch [141] have suggested that TPNH (or another reducing agent) is used to reduce a metal on the enzyme after its oxidation by reaction with oxygen Purification and study of the enzymes will undoubtedly yield more enlightening results in the future

With regard to the question of intermediates one of the earliest hypotheses [142] was that an unsaturated steroid was formed prior to the addition of oxygen but this idea has been quite effectively ruled out Another interesting suggestion [143] has been that a steroid hydroperoxide is formed which is subsequently reduced by TPNH to the required hydroxy product Failure to detect such intermediates [144] suggests that this is not the case but the possibility cannot be definitely excluded The most likely reaction on the basis of studies by Corey and White [138] is direct attack by a positively charged (electrophilic) oxygen on the appropriate carbon atom without formation of a steroid intermediate The site of the hydroxylation of course would be controlled by a specific enzyme Preliminary evidence [145] suggests that at least in steroid 11β hydroxylation there are several substrate specific enzymes which determine which of several 11-deoxy substrates 11-deoxycorticosterone (cortexone) or 11-deoxy 17 hydroxycorticosterone (cortexolone) will be hydroxylated

## BIOCHEMICAL ASPECTS OF THE ADRENOGENITAL SYNDROME

The adrenogenital syndrome can be distinguished from other forms of pseudohermaphroditism and virilism by two features (1) diffuse adrenocortical hyperplasia and (2) large quantities of androgenic 17 ketosteroids in the urine Initially treatment was directed toward these abnormalities Partial adrenalectomy often resulted in improvement but almost invariably there was progression of the disease as the remaining adrenal cortex hypertrophied Because of the androgen excess treatment with nonandrogenic 17 ketosteroids and estrogens was tried but without success

The first advance in therapy was reported by Bartter et al [146] and Wilkins et al [147] in 1950 Wilkins administered cortisone to a female pseudohermaphrodite with virilizing adrenal hyperplasia and found that

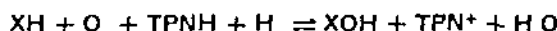
groups (two at position 4 and one at 14) are first hydroxylated and then eliminated as carbon dioxide There is also presumptive evidence that the 5–6 double bond in cholesterol is introduced through hydroxylation *of either C 5 or C 6 with subsequent removal of water*

In the series of reactions leading from cholesterol to the cortical hormones hydroxylation is essential, since removal of the cholesterol side chain probably proceeds by way of at least one hydroxylated intermediate 20 hydroxycholesterol Progesterone once formed is converted to cortisol by three additional hydroxylations at 11 17, and 21 Androgen biosynthesis from 17 hydroxyprogesterone also involves a hydroxylation reaction Many microorganisms, particularly the fungi [*128*] have the ability to hydroxylate steroids at almost all positions

In addition to the steroids many other reagents are hydroxylated In the following discussion no distinction will be made between aromatic and steroid hydroxylation Support for a common mechanism in both comes from the fact that various nonenzymatic ' model systems [*129, 130*] can promote the hydroxylation of both types of substrates [*131, 132*]

Several features characterize all the reactions studied thus far The first (shown by the use of isotopic oxygen e g [*134*]) is that the oxygen which becomes bound to the substrate is derived from atmospheric oxygen and not from the aqueous environment Hydroxylation is therefore an oxidation in the classic sense The second common finding is that a reducing agent, usually TPNH is required In a few cases [*134 135*] other reducing agents have been active

Enzymes which catalyze reactions of this type have been classified by Mason [*136*] as "mixed function oxidases The over all reaction can be generalized as follows



$$XH + O \;+ TPNH + H \rightleftharpoons XOH + TPN^{+} + H\;O$$

where XH = the substrate e g steroid to be hydroxylated

The biochemical and chemical aspects of this problem which have been investigated are (1) What sort of active oxygen is used? (2) At what stage is the reducing agent used? (3) Is the substrate ' activated " and *do any intermediates involving the substrate occur?*

With regard to the first of these the consensus based on experiments with model hydroxylating systems [*129*] and on Ruzicka s [*75*] speculations is that cationic oxygen ($O^{+}$) is the attacking reagent Recent results obtained by Bartlett [*137*] have suggested the way in which such an intermediate can be produced chemically and a series of biochemical and chemical experiments performed by Corey and various associates [*138*] has illustrated how such a reactant could attack steroids

The means by which reactive oxygen is produced biochemically is unknown Metal containing enzymes—possibly hemoproteins [*130*]—may

Pregnane-3α ol 11 20 dione
Pregnane 3α 17α diol 20-one [9] (17 hydroxypregnanolone)
Pregnane 3α 17α 20α triol 11 one (pregnanetriolone) [5]
Pregnane 3α 11β 17α 20α tetrol [1]
Pregnane-3α ol 20-one

In addition many urinary 17 ketosteroids are increased [*173 177 179 180 183 184 187*]

Androsterone [13]
Etiocholanolone [13]
11 Ketoetiocholanolone [11]
11β-Hydroxyandrosterone [12]
11 Ketoandrosterone [11]

11β Hydroxyetiocholanolone [1] has been reported both increased [*187*] and absent [*180*] The ratio of 11 oxygenated 17 ketosteroids to total 17 ketosteroids is consistently increased

The possibility of 21 deoxycorticoids resulting from the 21 dehydroxylation of hydrocortisone rather than from a primary defect in 21 hydroxylation has been considered and ruled out by Fukushima and Gallagher [*188*] $C^{14}$ Cortisol was administered to a patient with the adrenogenital syndrome and less than 0 5 per cent of the radioactivity was recovered in 21 deoxysteroids

*Pregnanetriol and Pregnanetriolone*

Excessive excretion of pregnanetriol [10] in the adrenogenital syndrome was first reported in 1937 by Butler and Marrian who noted its disappearance following partial adrenalectomy in one patient with this disease [*189*] It was also reported in the urine of virilized adults by others [*183 184*] and a simple test for it was devised in 1953 by Bongiovanni who then surveyed children with the adrenogenital syndrome and found increased amounts regularly present in their urine [*190 191*] Since pregnanetriol is the major corticosteroid metabolite in the urine of untreated patients it has become a valuable aid in establishing the diagnosis of the adrenogenital syndrome [*168*]

Pregnanetriolone [5] was first isolated from the urine of patients with the adrenogenital syndrome by Finkelstein and associates [*186*] As they [*186 192*] have pointed out the presence of this steroid in the urine may be of greater diagnostic significance than that of pregnanetriol It is always present in increased quantities in congenital adrenal hyperplasia and unlike pregnanetriol has been absent in the cases of adrenal carcinoma thus far examined

Treatment with cortisone or any of a variety of steroids which presumably inhibit the pituitary gland results in a prompt decline in urinary pregnanetriol and 17 ketosteroids [*168 190*]

urinary 17 ketosteroids which averaged 48 mg per day prior to treatment *fell on the second day, and after the ninth day averaged 6 5 mg per day* No change in the virilism was observed in the 15-day treatment period Since then there have been numerous reports of treatment with various corticosteroids [*8 11, 12 35 148–164*] which have suppressed the signs of the adrenogenital syndrome and permitted growth iso exual maturation and in the female ovulation and pregnancy

Bartter et al [*146 148*] found that ACTH stimulated the production of 17 ketosteroids in these patients but as had also been reported by Lewis and Wilkins in 1949 [*165*] did not produce an immediate $K^+$ loss or retention of $Na^+$ and $Cl^-$ in contrast to what occurs in normal individuals Cortisone administration on the other hand caused a decrease in 17 ketosteroids and was found to affect salt balance as in normal individuals but produced a more pronounced nitrogen loss

*The following outline, which has been of great value in understanding* the disea e was then propo ed by Bartter

1 The primary defect is the decreased production of the sugar hormone by the adrenal cortex

2 There is increased ACTH production because of failure of inhibition of ACTH secretion by 'sugar' hormones

3 Androgen production is increased

### DEFECTIVE HYDROXYLATION AT C-21

A more specific suggestion by Jailer in 1953 was that the hydroxylation of 17 hydroxyprogesterone at 11 and 21 to form hydrocortisone was impaired [*166*] In confirmation of these hypotheses all evidence now indicates that in the most common form of the syndrome there is at least a partial *block in the hydroxylation of C-21*

Blood 21 hydroxycorticosteroids [*154 167–172*] and urinary metabolites of hydrocortisone are decreased [*173*] If these potent inhibitors of ACTH secretion are diminished one would expect an increase in plasma ACTH This indeed was observed in 1953 by Sydnor and associates [*174*] The adrenal gland is therefore stimulated by excessive ACTH to produce large quantities of steroids which cannot be hydroxylated at C-21

The following urinary steroids none of which is hydroxylated at C-21 have been detected [*168 173 175–186*] in increased quantities in the adrenogenital syndrome [2]

Pregnane-3$\alpha$ 17$\alpha$ 20$\alpha$ triol (pregnanetriol) [10]
Allopregnane-3$\alpha$ 17$\alpha$ 20$\alpha$ triol [10]
Pregnane 3$\alpha$ 20$\alpha$ diol (pregnanediol) [4]

[2] In the following discussion of steroid metabolism the numbers in squares and superscript letters following names of steroids refer to Fig 20-14 where their structure and relationships are illustrated

The presence of the unusual urinary steroids can be largely explained by a defect in 21 hydroxylation the consequences of which are shown in Fig 20-14 These unusual steroids are products of alternate routes of metabolism of compounds whose major route is blocked in this syndrome For example the administration of 17α hydroxyprogesterone [8] to patients with this syndrome results in increased excretion of pregnanetriol [10] and 17 ketosteroids [*168*] It is likely that part of the increased 17 ketosteroids excreted in this syndrome is derived from 17α hydroxyprogesterone as shown in this figure Fukushima et al have demonstrated the conversion of deuterated 17α hydroxyprogesterone [8] to etiocholanolone [11] and androsterone [13] [*193*] and Jailer was able to simulate the urinary steroid pattern of congenital adrenal hyperplasia by the administration of 17α-hydroxyprogesterone [8] and 21 deoxycortisol [7] [*194*] Burstein et al [*195*] have reported the excretion of 3α 17α dihydroxy pregnane 11 20-dione [6] 11β-hydroxyetiocholanolone [12] and etiocholane 3α ol 11 17 dione [11] following the administration of 21 deoxycortisone [7] to a patient with rheumatoid arthritis Fukushima et al [*196*] have recently examined the radioactive metabolites following the administration of 21 deoxycortisol-4 $C^{14}$ to a normal subject and an untreated patient with congenital adrenal hyperplasia and found them to be similar This similarity indicates that the appearance of compounds such as pregnanetriolone [5] in the urine of individuals with the adrenogenital syndrome reflects the large quantities of 21 deoxycortisol [7] which

---

Fig 20-14 Pathways of corticoid synthesis in the adrenogenital syndrome Asterisks indicate urinary steroids that are abnormal or increased Metabolites of 21-deoxy cortisol [*196*] are marked with daggers and their arrangement is in part arbitrary e g compound 11 could arise from compound 7 via 5 6 or 12

- †1 5β-pregnane-3α 11β 17α 20α tetrol
- †1a 5β pregnane 3α 11β 17α 20β tetrol
- †2 3α 11β 17α-trihydroxy 5β pregnan 20-one
- 3 pregn-4-ene-3 20-dione (progesterone)
- 4 5β-pregnane 3α 20α diol (pregnanediol)
- †5 3α 17α 20α-trihydroxy 5β-pregnan 11-one (pregnanetriolone)
- †5a 3α 17α 20β trihydroxy 5β pregnan 11-one
- †6 3α 17α-dihydroxy 5β pregnan 11 20-dione
- 7 11β 17α-dihydroxypregn 4-ene 3 20-dione (21-deoxycortisol)
- 8 17α-hydroxypregn-4-ene-3 20-dione (17α hydroxyprogesterone)
- 9 3α 17α-dihydroxy 5β-pregnan 20-one
- 10 5β pregnane-3α 17α 20α-triol (pregnanetriol)
- 10 5α-pregnane-3α 17α 20α-triol
- †11 3α-hydroxy-5β androstan 11 17-dione
- †11a 3α-hydroxy-5α-androstan 11 17-dione
- †12 3α 11β-dihydroxy-5β-androstan 17-one
- †12a 3α 11β-dihydroxy 5α-androstan 17-one
- 13 3α-hydroxy-5β-androstan 17-one (etiocholanolone)
- 13a 3α-hydroxy-5α-androstan 17-one (androsterone)
- 14 17α 21-dihydroxypregn-4-ene-3 20-dione (compound S)
- 15 11β 17α 21 trihydroxypregn-4-ene-3 20-dione (compound F cortisol)
- 16 3α 11β 17α 21 tetrahydroxy-5β-pregnan 20-one (tetrahydrocortisol THF)

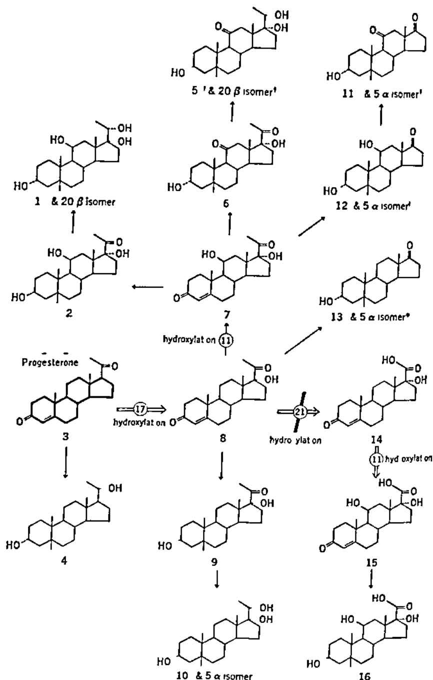



Fig 20 14 For descriptive legend see opposite page

These in vitro examinations of adrenal glands from two individuals with the adrenogenital syndrome support the concept of defective 21 hydroxylation and unimpaired 11 hydroxylation in this disease

## SALT LOSING FORM OF THE ADRENOGENITAL SYNDROME

In the group of patients characterized as salt losers no tetra hydrocortisol[16] is excreted and there is as a rule even more urinary pregnanetriol than in the previous group This indicates that the block in 21 hydroxylation is more nearly complete The adrenal gland even though overstimulated by ACTH is unable to produce enough 21 hydroxylated steroids to prevent salt loss and signs of adrenocortical insufficiency There is an intermediate group of patients who are able to produce reduced but apparently adequate amounts of hydrocortisone and who became salt losers only in the presence of superimposed stress such as infection [82]

An alternative explanation for the loss of salt is that there is a salt diuretic hormone produced by the overstimulated adrenal gland This speculation is due primarily to the observation that ACTH administration may cau e transient salt loss in this group of patients [29] Also Prader et al reported that aldosterone excretion was normal or elevated in six patients and concluded that salt loss is due to a sodium diuretic factor which in some patients is offset by increased aldosterone production [199] On the other hand Blizzard et al [200] have found that aldosterone is low in these patients even in the pre ence of sodium depletion

Recently two observations have supported the proposal that these patients produce a salt-excreting factor Klein and associates [?01] have described a substance obtained by paper chromatography of urine from the following sources premature infants following ACTH adminis tration infants with congenital adrenal hyperplasia with and without adrenocortical insufficiency and one patient with congenital adrenal hyperplasia being treated with corti one This material when injected into intact white rats caused excretion of sodium This did not occur with several known steroids including SC 5233 (see below) In addition Neher et al [20?] have isolated two urinary steroids $3\alpha$ $16\alpha$-dihydroxy pregnan 20-one and $3\alpha$ $16\alpha$-dihydroxyallopregnan 20-one from newborn infants a pregnant woman a patient with Cushing s syndrome and three patients with the salt losing form of the adrenogenital syndrome The first of these steroids is reported to cause salt loss in adrenalectomized male rats but data regarding this effect have not yet appeared [4] Although

[4] Neher et al Helvet chim acta 41 1667 1958 have found that $3\beta$ $16\alpha$-dihydroxy allopregnane 0-one i olated from hog adrenal promotes sodium loss in a irenal ect n iz d rat Coppage and Liddle J Cl n End crinol 20 ?9 1960 could not demon trate this effect in human subjects who received up to 400 mg per day of the steroid

accumulate "behind the block in 21 hydroxylation Pregnanetriolone[5] was found to be the chief metabolite of 21 deoxycortisol That 11 oxygenated 17-ketosteroids were found to represent less than 1 per cent of recovered steroid radioactivity was interpreted to mean that the large amounts usually found in the adrenogenital syndrome are mainly derived from $C_{19}$ precursors 17 Hydroxyprogesterone itself is probably only weakly androgenic [197] and the virilization characteristic of the disease is undoubtedly due to $C_{19}$ steroids such as androsterone which can be derived from 17 hydroxyprogesterone

Bartter suggested that although the adrenal gland appeared to be "insensitive' to ACTH, it might be stimulated by the increased secretion of ACTH to produce adequate "sugar hormone [146, 148] This in fact seems to be the case for most of the patients do not have adrenal insufficiency Their plasma contains 17 hydroxycorticosteroids and normal amounts of tetrahydrocortisone are excreted by some [8?] Further evidence that 21 hydroxylation is not totally impaired is the increase in blood and urine corticoids in some of these patients following ACTH administration [168]

In view of the enormous amounts of pregnanetriol and 17 ketosteroids excreted relative to decreased or normal amounts of tetrahydrocortisone one is led to the concept of an impaired conversion of 17α-hydroxyprogesterone[8] to hydrocortisone[15] In normal individuals this conversion is apparently quite efficient and only minute amounts of pregnanetriol[10] are excreted [191]

More direct evidence for the defect in 21 hydroxylation has been obtained by Bongiovanni [195] who examined adrenal glands from two individuals with the adrenogenital syndrome (one of whom had the salt losing form) These adrenal glands were found to contain neither of two 21 hydroxylated steroids compound S (17 hydroxy 11 deoxycorticosterone)[13], and cortisol[15] In sharp contrast two normal adrenal glands contained 4 to 6 μg per gm of tissue of each of these steroids Present in the diseased adrenal glands were large amounts of 17α hydroxyprogesterone[8] (118 and 155 μg per gm of tissue) compared with the amounts in normal adrenal glands (0 and 4 5 μg per gm) Homogenates of the diseased and normal glands were tested for their ability to hydroxylate 17-hydroxyprogesterone at C-21 Homogenates of two normal adrenal glands converted 10 per cent and 37 per cent of this substrate to 21 hydroxylated steroids (compounds S and F) Neither of these 21 hydroxylated products was formed in similarly treated homogenates of adrenal glands from two individuals with the adrenogenital syndrome When compared with homogenates of normal adrenal glands the ability of homogenates from two diseased glands to 11 hydroxylate 11 deoxy cortisol (compound S)[14] to cortisol[15] was not impaired and perhaps increased

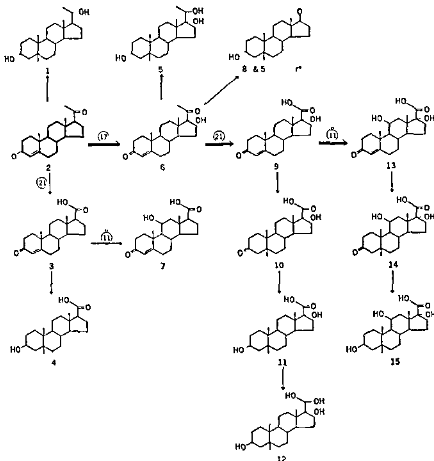



Fig 0-15 Defect in cort oid synthesis in the hyperten ive form of the adrenogenital yndrome \ terisks indi ate st roid identifi d in the uri e of a pati nt with this d sease [ *10*]

1 5$\beta$ pregn ne 3$\alpha$ 0$\alpha$-diol (pregnan hol)
2 pregn-4-ene-3 20-dione (prog terone)
3 1 hydroxypregn-4-ene 3 0-dione (DOC)
4 3$\alpha$ 1-dihydro y-5$\beta$ pregnan 0-one ( tetrahydro DOC)
5 5$\beta$ p egnane-3$\alpha$ 17$\alpha$ 0$\alpha$-triol (pregnan triol)
6 1 $\alpha$-hydroxypregn-4-ene-3 0-d one (1$\alpha$-hy dro yproge t ne)
11$\beta$ 1-dihydroxy pregn-4-ene-3 20-d one (compoun l B)
8 3$\alpha$-hydroxy 5$\beta$-androstan 17-one (etioch ol nolone)
9 3$\alpha$-hydroxy 5$\alpha$-andro tan 17-one (an dro teron )
9 1 $\alpha$ 21-d hydroxypregn-4-ene 3 20-d one (compo nd S)
10 1 $\alpha$ 1-dihydroxy-5$\beta$-pregnan-3 20-dione (dihydro S)
11 3$\alpha$ 1 $\alpha$ 1 trihydroxy-5$\beta$-pregnan 0-one (tetrahy dro S)
1 5$\beta$-pregnane-3$\alpha$ 17$\alpha$ 20 1 tetrol
13 11$\beta$ 1 $\alpha$ 1 trihy droxy pregn 4-en -3 20-di ne (compo nd F cortisol)
14 11$\beta$ 1 $\alpha$ 1 trihydroxy-5$\beta$-pregnan-3 0-d one ( dihy dro -cort sol)
15 3 11$\beta$ 1 $\alpha$ 1 tetrahydroxy 5$\beta$ pregnan 20-one ( tetrahydro -cortisol)

these observations do not yet permit a conclusion regarding the presence of a sodium-excreting steroid in these patients there is other experimental evidence for such a steroidal effect

Progesterone has been found to *inhibit the salt retaining effects of* deoxycorticosterone (DOC) in Addison s disease [203] A similar inhibition has been demonstrated in adrenalectomized rats [204] Cella and Kagawa [20a] have synthesized 3 (3-oxo-17β-hydroxy-4 androsten-17α ol) propionic acid γ lactone (SC 5233) and its 19-nor analogue (SC-8109) and have shown them to be more active in this regard than progesterone by factors of 8 and 27 Kagawa and associates [206] and Liddle [207] have shown that these two synthetic compounds can inhibit the action of aldosterone in rat and man respectively These observations indicate that salt metabolism can be compromised by a progesterone like compound Further experiments will determine the possible role of such a relationship in the salt losing form of the adrenogenital syndrome

## THE HYPERTENSIVE FORM

The defect in steroid metabolism associated with the hypertensive form of the adrenogenital syndrome is apparently different from that in the major syndrome This is inferred from the findings of Eberlein and Bongiovanni [208–210] who studied an 8 year-old female pseudohermaphrodite with congenital adrenal hyperplasia and hypertension

The most striking finding* was the presence of large amounts of Reichstein s compound S[9] and tetrahydro S[11] in the blood In addition tetrahydro S was the predominant urinary steroid These compounds produce Porter Silber chromogens which are characteristic of 17 hydroxycorticoids and which are usually low in the syndrome Because the Porter Silber chromogens were not low there was some initial confusion prior to their identification as 11 deoxysteroids The 17 ketosteroids present in the urine were dehydroepiandrosterone androsterone[8] and etiocholanolone[8] In contrast to the findings in the major and salt losing forms of the syndrome no 11-oxygenated 17 ketosteroids were detected and there was a small amount of pregnanetriol[5] excreted relative to the 17 ketosteroids

It is seen in Fig 20-15 that the observed pattern of steroid metabolites in this form of the disease could be explained by a defect in the β hydroxylation of C-11 Tetrahydro S[11] is the reduced form of compound S[9] which ordinarily is hydroxylated at C-11 to form cortisol[13] Pregnane 3α 21 diol 20 one ( tetrahydro DOC)[4] which was also isolated from the urine of this patient is a metabolite of 11 deoxycorticosterone (DOC)[3] It is generally thought that deoxycorticosterone is responsible for the hypertension that characterizes this form of the syndrome

* Numbers in squares and superscript letters following steroids refer to corresponding steroids in Fig 20 15

except for elevated urinary 17 ketosteroids have not yet been reported and defects in 11 and 21 hydroxylation can be inferred only by analogy to those children with either the salt-losing or the hypertensive form of the disease

It is germane to this account that there exists in women a fairly well defined syndrome which has been termed an intermediate form of the adrenogenital syndrome In 1953 Jones et al [21] found that ovulation followed cortisone treatment in a number of women who had slightly elevated urinary 17 ketosteroids and anovulatory cycles Similar observations have been reported by others [161 213] In 1958 studies on 6 women with oligomenorrhea or amenorrhea anovulatory temperature curves high normal to elevated urinary 17 ketosteroids and urinary 'pregnane complex' were reported by Gold and Frank [24] The pregnane complex included pregnanetriol and pregnanediol Treatment with $\Delta^1$ cortisone and $\Delta^1$ cortisol was followed by menstruation ovulatory temperature cycles and depression of 17 ketosteroids and pregnane complex in five patients Two of these five became pregnant shortly after the steroid treatment began

The Stein Leventhal syndrome is characterized by oligomenorrhea or amenorrhea hirsutism infertility and bilateral polycystic ovaries Ovulation usually ensues following wedge resection of the ovaries A small number of these patients who do not respond to this procedure respond to cortisone therapy by resuming cyclic ovulatory menses

These individuals perhaps also belong to the intermediate adrenogenital syndrome Of the six patients reported by Gold four definitely had enlarged polycystic ovaries This is also of interest since polycystic ovaries occasionally occur in the adrenogenital syndrome Until there is more information regarding steroid metabolism in these patients the relation of these endocrine disturbances to the adrenogenital syndrome must remain conjectural

## GENETICS

The familial nature of the adrenogenital syndrome has long been recognized [214] Knudson [215] reported 11 cases in 8 families in 1951 In a review of the cases reported from 1852 to 1952 Bentinck found 100 cases in 43 families [216] Childs reported 76 affected children from a total of 181 in 56 families [217] As pointed out by these authors the distribution is consistent with an autosomal recessive gene which when homozygous produces the syndrome The fact that the disease affects a single generation is also consistent with a homozygous recessive character It has also been reported in identical twins [218 219]

One possible inconsistency is the great predominance of affected females Of the 100 cases in Bentinck s series 83 were probably female

### VARIATIONS AND UNEXPLAINED OBSERVATIONS

In the preceding discussion the major form of the adrenogenital syndrome has been associated with a defect in 21 hydroxylation and the hypertensive form has been linked with a defect in 11β-hydroxylation There is probably more variability in the degree and type of defect than was implied in the preceding remarks For instance pregnane-3α 17α 21 triol 20-one (tetrahydro S) has been detected in the urine of four adrenogenital individuals with the major syndrome without associated hypertension [*182* *211*] This steroid which lacks a hydroxyl at C-11 and is hydroxylated at C-21 would certainly not be expected to result from a defect in 21 hydroxylation the proposed error in the major syndrome

Dyrenfurth et al [*212*] have also described tetrahydro S in the urine of a normotensive female pseudohermaphrodite following ACTH administration A brother of this patient had the hypertensive form of the disease and also excreted compound S and tetrahydro S As has been shown it is not unusual for individuals with the major form of the adrenogenital syndrome to produce some 21 hydroxylated steroids but their inability to 11β hydroxylate compound S[9] to form cortisol[13] is unexpected It is of particular interest that two patients described by Birke et al [*211*] excreted 11β hydroxyandrosterone in spite of impairment of the 11β hydroxylation of $C_{21}$ steroids indicated by the excretion of tetrahydro S[11] (Fig 20 15) One of the patients described by Bergstrand et al [*182*] excreted not only tetrahydro S but also tetrahydrocortisone the other patient excreted 11 ketopregnanetriol in addition to tetrahydro S

There is now additional evidence that obscures the individual nature of the different forms of the syndrome Bartter and Pronove [*86*] have studied three infants with the adrenogenital syndrome associated with both adrenal insufficiency and hypertension If the salt losing form and the hypertensive form each represent independently inherited defects then their coexistence in a single individual would be extremely rare That two of the infants with salt loss and hypertension are siblings makes the coincidental occurrence of the two defects in one person even less likely A more satisfactory explanation would be a single genetically determined defect resulting in the absence of a cofactor or enzyme common to both 21 and 11 hydroxylation

There are several possible sites for a defect in 11β hydroxylation since three separate enzymes and a heat stable cofactor in addition to TPNH and oxygen are required [*140*] A comparable situation may exist with 21 hydroxylation but the enzyme(s) is less well defined Adrenal microsomes (from which no active soluble fraction has yet been prepared) TPNH and oxygen are necessary for the reaction [*91*]

That there is a combination of defects in hydroxylation in these children with the mixed syndrome is only speculative since steroid metabolites,

a 21 carbon steroid which following oxidation at C-3 and isomerization from a 5 to a 4 unsaturated compound is hydroxylated at 17 21 and 11 to form cortisol

3 Androgenic 19-carbon steroids most probably arise from C 21 steroids by removal of the two-carbon side chain at C-17

4 TPNH is required for several different reactions in steroid synthesis and ACTH apparently stimulates this synthesis through TPNH ACTH causes increased production of 3 5 adenylic acid which stimulates a step in the production of glucose 1 phosphate from glycogen Glucose 1 phosphate is isomerized to glucose 6-P which is oxidized to pentose with the production of 2 moles of TPNH

5 There are several clinical forms of the adrenogenital syndrome Each probably represents a variation in the location and degree of hydroxylation defect at C 11 or C 21

The defect at C 21 is most common and results in decreased 21 hydroxylated steroids The pituitary gland uninhibited by these steroids releases increased quantities of ACTH which stimulates the adrenal gland to synthesize excessive 21 deoxysteroids some of which are androgenic or converted to androgens It is these androgenic steroids that are responsible for the virilization of these children

6 When the defect in hydroxylation at C-21 is severe, failure of synthesis of C 21 hydroxysteroids may be virtually complete and the deficiency so great that renal salt retention is seriously impaired Infants with such defects are subject to severe salt wasting and may develop Addisonian like crises

7 When the defect involves primarily hydroxylation of C 11 one of the steroid metabolites present in increased amounts is a derivative of 11 deoxycorticosterone Children with a defect in C-11 hydroxylation may show a hypertensive form of the adrenogenital syndrome Presumably the hypertension is attributable to excessive production of 11 deoxycorticosterone

8 The pattern of inheritance of congenital adrenal hyperplasia is most satisfactorily explained by an autosomal recessive gene the several hydroxylation defects being separately inherited There are however inconsistencies in this explanation

## BIBLIOGRAPHY


1 Bulloch W and Sequira J H On the relation of the suprarenal capsule to the sexual organs Tr Path Soc London 56 189 1905

2 Ogston Ein Beitrag zur Kasuistik abnormer geschlechtlicher Entwickelung *Osterr Jahrb Pediat* p 181 1872

3 Apert M Dystrophies en relation avec des lésions de capsules surrénales hirsutisme et progeria Bull Soc péd at Paris xii 510 1910

4 [illegible] Alfred *Le Syndrome génito-surrénal [illegible]* Thèse de Paris No 2 [illegible]

pseudohermaphrodites as were 53 of the 76 individuals reported by Childs One explanation for this inequality is that females because of the striking genital malformations are more easily detected This has been supported by Childs finding similar numbers of affected males (9) and females (11) in families in which an individual already had been recognized as having the adrenogenital syndrome It has also been suggested that there is a higher prenatal mortality in males with the disease but total pregnancy losses of mothers of these individuals are not abnormal

Since the metabolic defects differ in the hypertensive and major forms of the syndrome it is not surprising that they appear to be inherited separately In a survey of 18 families containing at least 2 affected children both forms of the syndrome were not observed in a single family [217] However in a family reported by Birke et al [211] 1 child had the adrenal insufficiency and 2 the major form of the syndrome Dyrenfurth has reported 2 children with the adrenogenital syndrome the brother having the hypertensive variant and the sister the major form [212]

If the clinical appearance of the adrenogenital syndrome represents the homozygous state of the genetic defect then the parents of affected individuals must be heterozygous Childs found that the mean increment in pregnanetriol excretion was higher following the administration of ACTH to 20 parents of children with the adrenogenital syndrome than to 18 controls [217]

Examination of the urine of parents of a child with the adrenogenital syndrome revealed high normal 17 ketosteroids and high dehydroisoandrosterone in the mother and low normal 17 ketosteroids and low dehydroisoandrosterone in the father [220] The parents and three normal siblings of three children with the adrenogenital syndrome were found to have normal excretion of 17 ketosteroids and 17 hydroxy corticosteroids [211]

## SUMMARY

1 Congenital adrenal hyperplasia causes progressive virilization and in girls pseudohermaphroditism This syndrome results from defects in the biosynthesis of adrenocorticoids

2 Adrenocorticoids arise from cholesterol, which is synthesized from acetate The main compounds on the way to cholesterol from acetate are $\beta$ hydroxy $\beta$ methyl glutaric acid mevalonic acid isopentenyl pyrophosphate geranyl pyrophosphate and farnesyl pyrophosphate Two molecules of the latter condense to form squalene which then cyclizes to form lanosterol which is converted to cholesterol Most of the side chain of cholesterol a 27 carbon steroid is removed to form pregnenolone

31 Faber Harold K The adrenogenital syndrome salt lo ing type Case reports of two males with prolonged ob ervations ince birth Pediatrics 20 458 1957
32 Eberlein W R The salt losing form of congenital adrenal hyperplasia Pediatrics 21 667 1958
33 Iav rsen T Congenital adrenocorti al hyperplasia with disturbed electrolyte regulation dysadrenocorticism Pediatrics 16 875 1955
34 Shepard T F II and Clausen S W Case of adrenogenital syndrome with hypertension treated with cortisone Pediatrics 8 805 1951
35 Wilkins L Crigler J F Jr Silverman S H Gardner L I, and Migeon C J Further studies on the treatment of congenital adrenal hyperplasia with cortisone III The control of hypertension with cortisone with a discussion of the variations in the type of congenital adrenal hyperplasia and a report of a case with probable defect of carbohydrate regulating hormones J Clin Endocrinol 12 1015 1952
36 Bartter F C and Pronove P Personal communication
37 Fieser L F and Fieser M *Natural Products Related to Phenanthrene* 3d ed Reinhold New York 1949
38 Wursch J Huang R L and Bloch K The origin of the isooctyl side-chain of cholesterol J Biol Chem 195 439 1952
39 Cornforth J W Hunter G D and Popjak G Studies of cholesterol biosynthesis 1 Biochem J 54 590 1953
40 *Cornforth J W Hunter G D and Popjak G Studies of cholesterol biosynthesis* 2 Biochem J 54 597 1953
41 Cornforth J W Gore I Y and Popjak G Studies on the biosynthesis of cholesterol 4 Biochem J 65 94 1957
42 Schoenheimer R and Breusch F Synthesis and destruction of cholesterol in the organism J Biol Chem 103 439 1933
43 Rittenberg D and Schoenheimer R Deuterium as an indicator in the study of *intermediary metabolism XI J Biol Chem* 121 235 1937
44 Sonderhoff R and Thomas H Die enzymatische Dehydrierung der Trideutero essigsäure *Ann* 530 195 1937
45 Ottke R C Tatum E L Zabin I and Bloch K Isotopic acetate and isovalerate in the synthesis of ergosterol by Neurospora J Biol Chem 189 429 1951
46 Bloch K and Rittenberg D On the utilization of acetic acid for cholesterol *formation J Biol Chem* 145 625 1942
47 Little H N and Bloch K Studies on the utilization of acetic acid for the biological synthesis of cholesterol J Biol Chem 183 33 1950
48 Bloch K The biological synthesis of cholesterol Recent Prog Hormone Res 6 111 1951
49 Bloch K Über die Herkunft des Kohlenstoff Atoms 7 in Cholestrin ein Beitrag zur Kenntnis der Biosynthese der Steroide Helvet chim acta 36 1611 1953
50 *Srere P A Chaikoff I L and Dauben W G The in vitro synthesis of cholesterol from acetate by surviving adrenal cortical tissue* J Biol Chem 176 829 1948
51 Srere P A Chaikoff I L Treitman S S and Burkstein L S The extrahepatic synthesis of cholesterol J Biol Chem 182 629 1950
52 Lynen F Acetyl coenzyme A and the fatty acid cycle Harvey Lect 48 210 1953
53 *Berg P Acyl adenylates an enzymatic mechanism of acetate activation J* Biol Chem 222 991 1956
54 Berg P Acyl adenylates the synthesis and properties of adenyl acetate J Biol Chem 222 1015 1956
55 Rudney H The biosynthesis of β hydroxy-β methylglutaric acid J Biol Chem 227 363 1957
56 Lynen F Henning U Bublitz C Sorbo B and Rueff Kroplin L Der chemische Mechanismus der Acetessigsäurebildung in der Leber Biochem Z 330 269 1958
57 Ferguson J J Durr I F and Rudney H The biosynthesis of mevalonic acid Proc Nat Acad Sc (U S) 45 499 1959

5 Clynn E E The adrenal cortex its rests and tumors its relation to other ductless glands and especially to sex Quart J Med 5 157 1912
6 Bratrud T L and Thompson W H Congenital hyperplasia of the adrenals Staff Meet Bull Hosp Univ Minn 15 25 1943
7 Wilkins L Hyperadrenocorticism Pediatrics 3 533 1949
8 Wilkins L The diagnosis of the adrenogenital syndrome and its treatment with cortisone J Pediat 41 860 1952
9 Wilkins L Grumbach M M Van Wyk J J Shepard T H and Papadatos C *Hermaphroditism—classification diagnosis selection of sex and treatment* Pediatrics 16 287 1955
10 Gothman L and Sandblom P Virilism in girls four cases with adrenogenital syndrome of various degree Acta chir scandinav 111 299 1956
11 Grumbach M M and Wilkins L The pathogenesis and treatment of virilizing adrenal hyperplasia Pediatrics 17 418 1956
12 Wilkins L *The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence* 2d ed Charles C Thomas Springfield Ill 1957
13 Witschi E Nelson W O and Segal S J Genetic developmental and hormonal aspects of gonadal dysgenesis and sex inversion in man J Clin Endocrinol 17 737 1957
14 Moore K L Graham M A and Barr M L The detection of chromosomal sex in hermaphrodites from a skin biopsy Surg Gynec & Obst 96 641 1953
15 Moore K L and Barr M L Smears from the oral mucosa in the detection of chromosomal sex Lancet 2, 57 1955
16 *Marberger E Boccabella R A and Nelson W O Oral smear as a method of* chromosomal sex detection Proc Soc Exper Biol & Med 89 488 1955
17 Anger L E Zapata G C Ely R S Kelley V C Female pseudohermaphroditism with penile urethra report of an unusual case of congenital adrenal hyperplasia A M A Dis of Child 95 410 1958
18 Sobel E H Sniffen R C and Talbot N B The testis V Use of testicular biopsies in the differential diagnosis of precocious puberty Pediatrics 8 701 1951
19 Wilkins L Fleischmann W and Howard J E Macrogenitosomia precox associated with hyperplasia of the androgenic tissue of adrenal and death from corticoadrenal insufficiency Endocrinology 26 385 1940
20 *Perloff W H and Hadd H E* The adrenogenital syndrome virilizing type in an adult male a case report Am J M Sc 234 441 1957
21 Jones G E S Howard J E and Langford H The use of cortisone in follicular phase disturbances *Fertil & Steril 4 49 1953*
22 Jones H W and Jones G E S The gynecological aspects of adrenal hyperplasia and allied disorders Am J Obst & Gynec 68 1330 1954
23 Kappas A Pearson O H West C H and Callagher T F A study of idiopathic hirsutism a transitional adrenal abnormality J Clin Endocrinol 16 517 1956
24 Gold J J and Frank R The borderline adrenogenital syndrome an intermediate entity Am *J Obst & Gynec 75 1034 1958*
25 Butler A M Ross R A and Talbot N B Probable adrenal insufficiency in an infant J Pediat 15 831 1939
26 Thelander H E Congenital adrenal cortical insufficiency associated with macrogenitosomia J Pediat 29 213 1946
27 *Zuelzer W W and Blum A Jr Adrenocortical insufficiency* in infants with the adrenogenital syndrome a clinical and pathologic study of four cases J Pediat 35 344 1949
28 Kyle L H and Knop C Q Simulation of cardiac disease by adrenocortical failure in infants New England J Med 243 681 1950
29 Lewis R A Klein R and Wilkins L Congenital adrenal hyperplasia with pseudohermaphroditism and symptoms of Addison s disease J Clin Endocrinol 10 703 1950
30 Block W M and Tucker F C Female pseudohermaphroditism clinical report and autopsy *finding of a case with adrenal crisis* J Iowa M Soc 47 37 195

85 Staple E Lynn W S and Gurin S An enzymatic cleavage of the cholesterol side chain J Biol Chem 219 845 1956
86 Beyer K F and Samuels L T Distribution of steroid-3β-ol dehydrogenase in cellular structures of the adrenal gland J Biol Chem 219 69 1956
87 Wang V S and Talalay P Enzymic isomerization of Δ-3 ketosteroids Biochim et biophys acta 18 300 1955
88 Hechter O Conversion of cholesterol to steroid hormones in *Cholesterol* edited by R P Cook Academic Press Inc New York 1958
89 Sweat M L Enzymatic synthesis of 17 hydroxycorticosterone J Am Chem Soc 73 4056 1951
90 Plager J E and Samuels L T The conversion of progesterone to 17 hydroxy 11-desoxycorticosterone by fractionated beef adrenal homogenate J Biol Chem 211 21 1954
91 Ryan K J and Engel L L Hydroxylation of steroids at carbon 21 J Biol Chem 225 103 1957
92 Rao B F and Heard R D H Biogenesis of the corticosteroids II Arch biochim et biophys 66 504 1957
93 Slaunwhite W R Jr and Samuels L T Progesterone is a precursor of testicular androgens J Biol Chem 220 341 1956
94 Lynn W S and Brown R Mechanisms of in vitro steroid oxidation Bioch m et biophys acta 21 403 1956
95 Werbin H Plotz J LeRoy G V and Davis E M Cholesterol a precursor of estrone in vivo J Am Chem Soc 79 1012 1957
96 Heard R D H Jacobs R O Donnell V J Peron F G Saffran J C Solomon S S Thompson L M Willoughby H and Yates C H The application of C to the study of the metabolism of the sterols and steroid hormones Recent Progr Hormone Res 9 383 1954
97 Ryan K J Biological aromatization of steroids J Biol Chem 234 268 1959
98 Brady R O Biosynthesis of radioactive testosterone in vitro J Biol Chem 193 145 1951
99 Heard R D H Bligh E G Cann M C Jellinck P H O Donnell V J Rao B G and Webb J L Biogenesis of the sterols and steroid hormones Recent Progr Hormone Res 12 45 1956
100 Schotz M C Rice L I and Alfin-Slater R B Further studies on cholesterol in liver cell fractions of normal and cholesterol fed rats J Biol Chem 204 19 1953
101 Smith P E Ablation and transplantation of the hypophysis in the rat Anat Record 32 221 1926
102 Li C H Simpson M E Evans H M Isolation of adrenocorticotropic hormone from sheep pituitaries Science 96 450 1942
103 Sayers G White A and Long C N H Preparation and properties of adrenotropic hormone J Biol Chem 149 425 1943
104 Hechter O Zaffaroni A Jacobsen R P Levy H Jeanloz R W Schenker V and Pincus G The nature and the biogenesis of the adrenal secretory product Recent Progr Hormone Res 6 215 1951
105 Sayers G and Sayers M A The pituitary adrenal system Recent Progr Hormone Res 2 81 1948
106 Bell P H Purification and structure of β-corticotropin J Am Chem Soc 76 5565 1954
107 Howard K S Shepherd R G Eigner E A Davies D S and Bell P H Structure of β-corticotropin final sequence studies J Am Chem Soc 77 3419 1955
108 Li C H Geschwind I I Cole R D Raacke I D Harris J I and Dixon J S Amino acid sequence of alpha corticotropin Nature London 176 687 1955
109 Linderstrøm Lang K quoted in Li C H Hormones of the anterior pituitary gland Advances Protein Chem 11 10 1956
110 Harris J I and Roos P Amino acid sequence of melanophore stimulating peptide Nature London 178 90 1956

58 Wright L D Cleland M Dutta B N and Norton J S Mevaldic acid in the biosynthesis of mevalonic acid J Am Chem Soc 79 6572 1957
59 Lynen F Verzweigte Carbonsäuren als Baustoffe der Polyisoprenoide in *Proceedings of the International Symposium on Enzyme Chemistry* p 57 Tokyo 1957
60 Bucher N R McGarrahan K Gould E and Loud A V Cholesterol biosynthesis in preparations of liver from normal fasting x irradiated cholesterol fed triton or Δ-cholesten 3-one treated rats J Biol Chem 234 262 1959
61 Tavormina P A Gibbs M H and Huff J W The utilization of β-hydroxy β-methyl δ-valeralactone in cholesterol biosynthesis J Am Chem Soc 78 4498 1956
62 Cornforth J W and Popjak G Studies on the biosynthesis of cholesterol 3 Biochem J 58 403 1954
63 Cornforth J W Cornforth R H Popjak G and Gore I Y Studies on the biosynthesis of cholesterol 5 Biochem J 69 146 1958
64 Isler O Ruegg R Würsch J Gey K F and Pletscher A Zur Biosynthese des Cholestrins aus β-α-Dihydroxy β-methyl Valeriansäure Helvet chim acta 40 2369 1957
65 Tchen T T Mevalonic kinase purification and properties J Biol Chem 233 1100 1958
66 Chaykin S Law J Phillips A H Tchen T T and Bloch K Phosphorylated intermediates in the synthesis of squalene Proc Nat Acad Sc (US) 44 998 1958
67 Agranoff B W Eggerer H Henning U and Lynen F Isopentenol pyrophosphate isomerase *J Am Chem Soc 81 1254 1959*
68 Rilling H Tchen T T and Bloch K On the mechanism of squalene biogenesis Proc Nat Acad Sc (US) 44 167 1958
69 Lynen F Eggerer H Henning U and Kessel I Farnesyl pyrophosphat und 3-methyl $\Delta^3$ butenyl 1 pyrophosphat die biologischen Vorstufen des Squalens Angew Chem 70 738 1958
70 Heilbron I M Kamm E David and Owens W M The unsaponifiable matter from the oils of elasmobranch fish Part 1 *J Am Chem Soc* 48 1630 1926
71 Channon H J The biological significance of the unsaponifiable matter of oils Biochem J 20 400 1926
72 Robinson R Structure of cholesterol J Soc Chem Ind London 53 1062 1934
73 Woodward R B and Bloch K The cyclization of squalene in cholesterol synthesis J Am Chem Soc 75 2023 1953
74 Clayton R B and Bloch K The biological conversion of lanosterol to cholesterol J Biol Chem 218 319 1956
75 Ruzicka L The isoprene rule and the biogenesis of terpenic compounds Experientia 9 357 1953
76 Tchen T T and Bloch K On the mechanism of enzymatic cyclization of squalene J Biol Chem 226 931 1957
77 Olson J A Jr Lindberg M and Bloch K On the demethylation of lanosterol to cholesterol J Biol Chem 226 941 1957
78 Gautschi F and Bloch K *On the structure of an intermediate in the biological* demethylation of lanosterol J Am Chem Soc 79 684 1957
79 Gautschi F and Bloch K Synthesis of isomeric 4 4-dimethyl cholesterols and identification of a lanosterol metabolite J Biol Chem 233 1343 1958
80 Johnston J D and Bloch K In vitro conversion of zymosterol dihydrozymosterol to cholesterol J Am Chem Soc 79 1145 1957
81 Bloch K The biological conversion of cholesterol to pregnanediol J Biol Chem 157 661 1945
82 Werbin H Chaikoff I L and Jones F Isolation of H-cortisol from the urine of guinea pigs fed H³-cholesterol J Biol Chem 234 28- 1959
83 Zaffaroni A Hechter O and Pincus G Adrenal conversion of $C^{14}$ labeled cholesterol and acetate to adrenal cortical hormones J Am Chem Soc 73 1390 1951
84 Lynn W S Jr Staple E and Gurin S Catabolism of cholesterol by in vitro systems Fed Proc 14 783 1955

137 Bartlett P D and Storrey B T Ionic fission of the O—O bond in t butyl arylpersulfonates J Am Chem Soc 80 4954 1958
138 Corey E J and White R W Cationic displacement of hydrogen by oxygen at a saturated carbon atom J Am Chem Soc 80 6686 1958
139 Kaufman S The participation of tetrahydrofolic acid in the enzymic conversion of phenylalanine to tyrosine Biochim et biophys acta 27 428 1958
140 Tomkins G M Curran J F and Michael P J Further studies on enzymic adrenal 11 β-hydroxylation Biochim et biophys acta 28 449 1958
141 Tchen T T and Bloch K Role of oxygen and reduced pyridine nucleotides in the biogenesis of cholesterol Abstract Am Chem Soc Meeting September 1956 p 56c
142 Hayano M and Dorfman R I On the mechanism of the C-11 β-hydroxylation of steroids J Biol Chem 211 227 1954
143 Grant J K The in vitro enzymatic hydroxylation of steroids Biochem J 64 559 1956
144 Tomkins G M Studies on the mechanism of steroid hydroxylation *Proceedings of the 4th International Congress on Biochemistry* Vienna September 1958
145 Tomkins G M Michael P J and Curran J F Studies on the nature of steroid 11 β-hydroxylation Biochim et biophys acta 23 655 1957
146 Bartter F C Forbes A P and Leaf A Congenital adrenal hyperplasia associated with the adrenogenital syndrome an attempt to correct its disordered hormonal pattern J Clin Invest 29 797 1950
147 Wilkins L Lewis R A Klein R and Rosemberg E The suppression of androgen secretion by cortisone in a case of congenital adrenal hyperplasia Bull Johns Hopkins Hosp 86 249 1950
148 Bartter F D Albright F Forbes A P Leaf A Dempsey E and Carroll E The effects of adrenocorticotropic hormone and cortisone in the adrenogenital syndrome associated with congenital adrenal hyperplasia an attempt to explain and correct its disordered hormonal pattern J Clin Invest 30 237 1951
149 Wilkins L Lewis R A Klein R Gardner L I Crigler J F Jr Rosemberg E and Migeon C J Treatment of congenital adrenal hyperplasia with cortisone J Clin Endocrinol 11 1 1951
150 Crigler J F Jr Silverman S H and Wilkins L Further studies on the treatment of congenital adrenal hyperplasia with cortisone IV Effect of cortisone and compound B in infants with disturbed electrolyte metabolism Pediatrics 10 397 1952
151 Gardner L I and Migeon C J Urinary dehydroisoandrosterone in hyperadrenocorticism influence of cortison hydrocortisone and ACTH J Clin Endocrinol 12 1117 1952
152 Wilkins L Crigler J F Jr Silverman S H Gardner L I and Migeon C J Further studies on the treatment of congenital adrenal hyperplasia with cortisone II The effects of cortisone on sexual and somatic development with an hypothesis concerning the mechanism of feminization J Clin Endocrinol 12 [illegible]7 1952
153 Wilkins L Gardner L I Crigler J F Jr Silverman S H and Migeon C J Further studies on the treatment of congenital adrenal hyperplasia with cortisone I Comparison of oral and intra-muscular administration of cortisone with a note on the suppressive action of compounds F and B on the adrenal J Clin Endocrinol 12 257 1952
154 Kelley V C Ely R S and Raile R B Metabolic studies in patients with congenital adrenal hyperplasia Effects of cortison therapy J Clin Endocrinol 12 1140 19[illegible]
155 [illegible] Logan C B Albert A and M[illegible] H I Use of cortisone for congenital adrenal hyperplasia in the [illegible] report of a case J Clin Endocrinol 13 [illegible] 1953
156 Wilkins L and Cara J [illegible] Further studies on the treatment of congenital adrenal hyperplasia with cortisone V Effects of cortisone on testicular development J Clin Endocrinol 14 287 1954
157 Jailer J W and Wallace E Z The effect of intra[illegible] administered hydro-

111 Geschwind I I Li C H and Barnafi L The structure of the β-melanocyte stimulating hormone J Am Chem Soc 79 620 1957
112 Dixon H B The melanophore-stimulating activity of corticotropin Biochim et biophys acta 19 392 1956
113 Boissonnas R A St Guttmann Waller J I and Jaquenoud P A Synthesis of a polypeptide with ACTH like structure Experientia 12 446 1956
114 Boissonnas R A *Proceedings of the Brook Lodge Symposium on Protein Hormones and Biologically Active Peptides* Kalamazoo Mich October 1958
115 Hofmann Klaus *Proceedings of the Brook Lodge Symposium on Protein Hormones and Biologically Active Peptides* Kalamazoo Mich October 1958
116 Sutherland E W and Wosilait W D The relationship of epinephrine and glucagon to liver phosphorylase I Liver phosphorylase preparation and properties J Biol Chem 218 459 1956
117 Wosilait W D and Sutherland E The relationship of epinephrine and glucagon to liver phosphorylase II Enzymatic inactivation of liver phosphorylase J Biol Chem 218 469 1956
118 Rall T W Sutherland E W and Wosilait W D The relationship of epinephrine and glucagon to liver phosphorylase III Reaction of liver phosphorylase in slices and extracts J Biol Chem 218 483 1956
119 Wosilait W D Studies on the organic phosphate moiety of liver phosphorylase J Biol Chem 233 597 1958
120 Rall T W Sutherland E W and Berthet J The relationship of epinephrine and glucagon to liver phosphorylase IV Effect of epinephrine and glucagon on the reactivation of phosphorylase in liver homogenates J Biol Chem 224 463 1957
121 Rall T W and Sutherland E W Formation of a cyclic adenine ribonucleotide by tissue particles J Biol Chem 232 1065 1958
122 Sutherland E W and Rall T W Fractionation and characterization of a cyclic adenine ribonucleotide formed by tissue particles J Biol Chem 232 1077 1958
123 Haynes R C Jr and Berthet L Studies on the mechanism of action of the adrenocorticotropic hormones J Biol Chem 225 115 1957
124 Haynes R C The activation of adrenal phosphorylase by the adrenocorticotropic hormone J Biol Chem 233 1220 1958
125 Kelly T J Nielson E D Johnson R B and Vestling C S Glucose 6-phosphate dehydrogenase of adrenal tissue J Biol Chem 212 545 1955
126 Sweat M L and Lipscomb M D A transhydrogenase and reduced triphosphopyridine nucleotide involved in the oxidation of desoxycorticosterone by adrenal tissue J Am Chem Soc 77 5185 1955
127 Harris G W Neural control of the adenohypophysis J Physiol 107 418 1948
128 Peterson D H Microbiological conversions of steroids Recent Chem Progr 17 211 1956
129 Udenfriend S Clark C T Axelrod J and Brodie B B Ascorbic acid in aromatic hydroxylation J Biol Chem 208 731 1954
130 Mason H S Onopryenko I and Buhler D Hydroxylation the activation of oxygen by peroxidase Biochim et biophys acta 24 225 1957
131 Mason H S Mechanisms of oxygen metabolism Advances Enzymol 19 79 1957
132 Cier A Nofre C and Revol A Hydroxylation de la 11-desoxycorticosterone par le système modèle oxygène ion ferreux ascorbate Compt rend acad sci Roumanie 247 542 1958
133 Hayano M Lindberg M C Dorfman R I Hancock J E H and Doering W Von E On the mechanisms of the C-11 β hydroxylation of steroids a study with $H_2O^{18}$ and $O_2^{18}$ Arch Biochem and Biophys 59 529 1955
134 Mitoma C Studies on partially purified phenylalanine hydroxylase Arch Biochem Biophys 60 476 1956
135 Tabor H T and Hayaishi T Enzymatic formation of formyl a partic acid from imidazole acetic acid J Am Chem Soc 76 5570 1954
136 Mason H S Mechanisms of oxygen metabolism Science 125 1185 1957

cortical hyperplasia report of a case with studies of the excretion of steroid metabolites J Clin Endocrinol 15 430 1957
180 Masuda M Urinary ketosteroid excretion patterns in congenital adrenal hyperplasia J Clin Endocrinol 17 1181 1957
181 Fukushima D K and Gallagher T F Steroid isolation studies in congenital adrenal hyperplasia J Biol Chem 229 85 1957
182 Bergstrand C G Birke G and Plantin L O The corticosteroid excretion pattern in infants and children with the adrenogenital syndrome Acta endocrinol 30 500 1959
183 Mason H L and Kepler E J Isolation of steroids from the urine of patients with adrenal cortical tumors and adrenal cortical hyperplasia a new 17 ketosteroid androstane-3α-11-diol 17-one J Biol Chem 161 235 1945
184 Miller A M and Dorfman R I Metabolism of the steroid hormones—isolation of 13 steroid metabolites from a patient with (probable) adrenal hyperplasia Endocrinology 46 514 1950
185 Cox R I and Finkelstein M Pregnane 3α 17α 20α triol and pregnane 3α 17α 20α triol 11-one excretion by patients with adrenogenital dysfunction J Clin Invest 36 1726 1957
186 Finkelstein M Von Euw H and Reichstein T Isolierung von 3 alpha 17 20-tri-oxy pregnanon (11) aus pathologischem Harn Helvet chim acta 36 1266 1953
187 Brooks R V A method for the quantitative fractionation of urinary 17-oxo steroids with some observations on steroid excretion during administration of ACTH and in the adrenogenital syndrome Biochem J 68 50 1958
188 Fukushima D K and Gallagher T F Absence of 21 dehydroxylation in congenital adrenal hyperplasia J Clin Endocrinol 18 694 1958
189 Butler G C and Marrian G F The isolation of pregnane 3 17 20 triol from the urine of women showing the adrenogenital syndrome J Biol Chem 119 565 1937
190 Bongiovanni A M The detection of pregnandiol and pregnantriol in the urine of patients with adrenal hyperplasia Suppression with cortisone Bull J Hopkins Hosp 92 244 1953
191 Bongiovanni A M and Clayton G W Jr A simplified method for the routine determination of pregnanediol and pregnanetriol in urine Bull J Hopkins Hosp 94 180 1954
192 Finkelstein M and Shoenberger J Urinary steroids in 10 cases of adrenal carcinoma with special reference to pregnane-3α 17α 20α triol 11-one J Clin Endocrinol 19 608 1959
193 Fukushima D K Dobriner K and Gallagher T F Metabolic studies with deuterium steroid hormones Fed Proc 10 185 1951
194 Jailer J W Gould J J Vande Wiele R and Lieberman S 17α hydroxy progesterone and 21-desoxyhydrocortisone their metabolism and possible role in congenital adrenal virilism J Clin Invest 34 1639 1955
195 Burstein S Savard K and Dorfman R I The in vivo metabolism of [illegible] desoxycortisone Endocrinology 53 267 1953
196 Fukushima D K Bradlow H L Hellman L and Gallagher T F Further studies of 21-deoxyhydrocortisone-4 C in man J Clin Endocrinol 19 [illegible] 19[illegible]
197 Pfiffner J J and North H B 17β-Hydroxyprogesterone J Biol Chem 132 459 1940
198 Bongiovanni A M In vitro hydroxylation of steroids by whole adrenal homogenates of beef normal man and patients with the adrenogenital syndrome J Clin Invest 37 134[illegible] 1958
199 Prader A Spahr A and Neher R Erhöhte Aldosteronausscheidung beim kongenitalen adrenogenitalen Syndrom ein Beitrag zur Pathogenese des Syndroms Schweiz Med Wchnschr 86 108[illegible] 19[illegible]
200 Blizzard R M Liddle G W and Migeon C J Aldosterone excretion in patients with virilizing adrenal hyperplasia with normal and low-salt intake The Endocrinology Society program of the 40th Meeting p 60 June 1958

cortisone on the urinary 17 ketosteroids in patients with adrenal virilism Ann New York Acad Sc 61 44? 1955
158 Spence A W Jenkins J S and Robinson A M Adrenal virilism treated with steroids Brit M J 2 1348 1956
159 Smith E K and DeAlvarez R R Congenital adrenal hyperplasia (pseudohermaphroditism) before and after cortisone therapy I Adolescent development and menstruation Am J Obst & Gynec 72 404 1956
160 Skoog W A and Adams W S Metabolic balance study in a case of virilizing adrenal hyperplasia treated with prednisone J Clin Endocrinol 17 364 1957
161 DeAlvarez R R and Smith E K Congenital adrenal hyperplasia endocrine patterns in women and effects of cortisone treatment Obst & Gynec 9 476 1957
162 Blizzard R M and Wilkins L Present concepts of steroid therapy in virilizing adrenal hyperplasia A M A Arch Int Med 100 729 1957
163 Bongiovanni A and Eberlein W R Experience with use of 6-methylprednisolone in the adrenogenital syndrome Metabolism 7 457 1958
164 Jenkins D and Schemmel J L Metabolic effects of 6-methylprednisolone Metabolism 7 416 1958
165 Lewis R A and Wilkins L The effect of adrenocorticotropic hormone in congenital adrenal hyperplasia with virilism and in Cushing s syndrome treated with *methyl testosterone J Clin Invest 28 394 1949*
166 Jailer J W Virilism Bull New York Acad Med 29 377 1953
167 Bayliss R I S Broadbent I E and Steinbeck A W Congenital adrenal hyperplasia effects of corticotropin and tetrahydrocortisone Lancet 1 431 1954
168 Bongiovanni A M Eberlein W R and Cara J Studies on the metabolism of adrenal steroids in the adrenogenital syndrome J Clin Endocrinol 14 409 1954
169 Bongiovanni A M Defective steroidal biogenesis in congenital adrenal hyperplasia adrenogenital syndrome uncomplicated and hypertensive forms Pediatrics 21 661 1958
170 Ely R S Raile R B Bray P F and Kelley V C Studies of the 17 hydroxycorticosteroids IV Evaluation of a standard ACTH 17 hydroxycorticosteroid response test in children Pediatrics 13 403 1954
171 Perkoff G T Sandberg A A Nelson D H and Tyler F H Clinical usefulness of determination of circulating 17 hydroxycorticosteroid levels A M A Arch Int Med 93 1 1954
172 Wallace E Z Christy N P and Jailer J W Clinical application of the simplified Silber Porter method for determining plasma 17 hydroxycorticosteroids J Clin Endocrinol 15 1073 1955
173 Eberlein W and Bongiovanni A Partial characterization of urinary adrenocortical steroids in adrenal hyperplasia J Clin Invest 34 1337 1955
174 Sydnor K L Kelley V C Raile R B Ely R S and Sayers G Blood adrenocorticotropin in children with congenital adrenal hyperplasia Proc Soc Exper Biol & Med 82 695 1953
175 Venning E H Weil P G and Browne J S L Excretion of sodium pregnandiol glucuronidate in the adrenogenital syndrome Proc Am Soc Biol Chemists J Biol Chem 128 CVII CVIII 1939
176 Genitis V E and Bronstein I I Pregnandiol excretion in female pseudohermaphroditism (? congenital adrenal hyperplasia) studies in two cases J A M A 119 704 1942
177 Bergstrand C G Birke G Plantin L O and Zetterstrom R The adrenogenital syndrome in children a clinical and steroid metabolic study Acta endocrinol 16 210 1954
178 Bergstrand C G and Gemzell C A Pregnanediol excretion in normal children and in children with various endocrine disorders including congenital adrenal hyperplasia J Clin Endocrinol 17 870 1957
179 Bush I E Swyer G I M Stern M I and Willoughby M L N Adreno-

Part Six

# Diseases of Purine and Pyrimidine Metabolism

201 Klein R Taylor I Papadatos C Laron Z Keele D Fortunato J Byers C and Billings C Sodium losing material in human urine Proc Soc Exper Biol & Med 98 863 1958
202 Neher R Meystre C and Wettstein A Neue 16α Hydroxysteroide aus menschlichem Urin und aus Schweine Nebennieren Isolierung Konstitution Synthesen Helvet chim acta 42 132 1959
203 Landau R L Bergenstal D M Lugibihl K and Kascht M L The metabolic effects of progesterone in man J Clin Endocrinol 15 1194 1955
204 Kagawa C M Blocking urinary electrolyte effects of desoxycorticosterone with progesterone in rats Proc Soc Exper Biol & Med 99 705 1958
205 Cella J A and Kagawa C M Steroidal lactones J Am Chem Soc, 79 4808 1957
206 Kagawa C M Cella J A and Van Arman C G Action of new steroids in blocking effects of aldosterone and deoxycorticosterone on salt Science 126 1015 1957
207 Liddle G W Sodium diuresis induced by steroidal antagonists of aldosterone Science 126 1016 1957
208 Eberlein W R and Bongiovanni A M Congenital adrenal hyperplasia with hypertension unusual steroid pattern in blood and urine J Clin Endocrinol 15 1531 1955
209 Bongiovanni A M and Eberlein W R Clinical and metabolic variations in the adrenogenital syndrome Pediatrics 16 628 1955
210 Eberlein W R and Bongiovanni A M Plasma and urinary corticosteroids in the hypertensive form of congenital adrenal hyperplasia J Biol Chem 223 85 1956
211 Birke G Diczfalusy E Plantin L-O Robbe H and Westman A Familial congenital hyperplasia of the adrenal cortex Acta endocrinol 29 55 1958
212 Dyrenfurth I Sybulski S Notchev V Beck J C and Venning E H Urinary corticosteroid excretion patterns in patients with adrenocortical dysfunction J Clin Endocrinol 18 391 1958
213 Greenblatt R B Barfield W E and Lampros C P Cortisone in the treatment of infertility Fertil & Steril 7 203 1956
214 Jacobziner H and Gorfinkel A Familial congenital adrenal cortical syndrome Am J Dis Child 52 308 1936
215 Knudson A G Jr Mixed adrenal disease of infancy J Pediat 39 408 1951
216 Bentinck R C Hinman F Sr Lisser H and Traut H F The familial congenital adrenal syndrome report of two cases and review of the literature Postgrad Med 11 301 1952
217 Childs B Grumbach M M and Van Wyk J J Virilizing adrenal hyperplasia a genetic and hormonal study J Clin Invest 35 213 1956
218 Wolff S Female pseudohermaphroditism with adrenocortical failure in identical twins Arch Dis Childhood 29 132 1954
219 Schneeberg N G Steinberg A Malen M M Chernoff B and Yap C L Congenital virilizing adrenal hyperplasia in identical twins J Clin Endocrinol 19 203 1959
220 Jensen C C and Björklund S-I The adrenogenital syndrome discussion of a case with special reference to the paternal and maternal steroid patterns Acta endocrinol 22 42 1956

# Chapter 21

# Gout*

*James B Wyngaarden*

## HISTORICAL CONSIDERATIONS

Gout is a disease rich in historical lore Its descriptions can be traced to the dawn of medical literature Ancient Greek and Roman physicians possessed an intimate knowledge of its varied features Over the centuries the differentiation of gout from other articular disorders became clouded and it remained for Thomas Sydenham (1683) to reemphasize this distinction [1] His masterly description of the disease based in part upon his 34 years of personal affliction marks the beginning of the modern clinical history of gout

The chemical history of gout began a century after Sydenham when in 1776 Scheele [2] discovered uric acid as a constituent of a kidney stone Shortly thereafter Wollaston (1797) and Pearson (1798) demonstrated urates in the tophi of patients with gout [3 4] Another 50 year later A B Garrod performed his historic experiment in which he demonstrated first by the murexide test [5] and later by his famous thread test (1854) [6] an increased amount of uric acid in the blood of gouty subjects

At this time the structure of uric acid was unknown With the establishment by Emil Fischer [7] in 1898 that uric acid was a purine compound its potential relationship to the nucleic acid constituents adenine and guanine was appreciated and a key role of purine metabolism in the pathophysiology of gout was recognized With the introduction of a reliable method for determination of uric acid in blood by Folin and Denis in 1913 [8] clinical and metabolic studies in gout were greatly facilitated A valuable introduction to the history of gout is that of Hartung [9]

*Studies of the author cited in this chapter were supported in part by grant A 1391 from The National Institutes of Health Public Health Service

Table 21 1 are cited five representative studies employing three types of methods now in common use Colorimetric methods such as those of Folin [16] or Benedict [17] are affected by loss of urate in the protein precipitation step by nonspecificity of color production and by inhibitory and augmentatory influences These opposing errors cancel each other to a large extent for routine diagnostic use these or similar methods are quite satisfactory The upper limit of normal (M + 2 s d ) for males is about 6 3 mg per 100 ml [12] for females about 5 8 mg per 100 ml

In order to obviate errors of nonspecificity colorimetric analyses for uric acid may be performed before and after incubation of the sample with uricase The values obtained by this procedure are regarded as true uric acid On an unrestricted diet the upper limits of normal are 7 8 and 5 9 mg per 100 ml in males and females respectively [13]

Currently the preferred method is the direct enzymatic differential spectrophotometric method of Kalckar [18] as modified by Praetorius [19] This method involves measurement of the change in absorbance at 292 mμ of a diluted sample of serum when treated with purified uricase at pH 9 4 The method is sensitive accurate and specific and it avoids errors inherent in colorimetric methods requiring precipitation of proteins and involving some degree of nonspecificity in color development By this procedure normal values (2 s d level of significance) extend to 7 5 and 5 8 mg per 100 ml for males and females respectively [14 15]

The sex difference in urate levels is found with all methods but varies in magnitude in different studies The mean male to female ratio in 10 published studies employing a variety of analytic methods is 1 19 [13 20] In children there is normally no sex difference in serum urate levels which are lower than in adults After puberty the levels rise in both sexes but more so in males so that there is thereafter a higher mean level in males than in females These differences have been attributed to higher renal clearances of urate in children than in adults and among adults in women than in men [21 22] Possible differences of urate production have not been assessed Finally there is a significant correlation between serum uric acid level and surface area in normal men ($r = 0.427$ $p = 0.001$) but not in normal women ($r = 0.187$ $p > 0.1$) [13] Smyth Stecher and Wolfson [23] found that urate levels rose in normal women after the menopause to equal those of men but Hauge and Harvald [15] found the sex difference in their control subjects was lifelong The mean value was only slightly lower in women under 50 years than in those over this age

Despite the obvious difficulties of defining valid precise limits to the normal range of serum uric acid value clinical experience has led physicians to view any value found by a colorimetric method in excess of

Many authors have compiled rosters of distinguished personages in history who have had gout The list is impressive and includes persons of such diverse claims to fame as the Medici Isaac Newton Charles Darwin, Martin Luther, John Calvin Benjamin Franklin Ben Jonson Samuel Johnson and Goethe [10]

## DEFINITIONS

Primary gout is a genetically determined disorder of purine metabolism in which the cardinal feature is hyperuricemia The hyperuricemia is initially asymptomatic and may remain so but in a small percentage of affected individuals the disorder is complicated by episodes of acute arthritis of unknown cause by chronic arthritis due to depositions of urate in cartilage and other tissues by formation of renal calculi composed of uric acid and possibly by an accelerated development of vascular and renal disease

Asymptomatic hyperuricemia is considerably more common than overt gout Most students of this disease currently regard asymptomatic hyperuricemia as synonomous with the gouty diathesis and tacitly assume the potentiality for symptomatic gout in each hyperuricemic individual

A large section of this chapter is devoted to an inquiry into the cause or causes of hyperuricemia in primary gout Although recent studies have greatly increased understanding of the general nature of one metabolic derangement present in certain patients with gout the precise biochemical defect or defects leading to hyperuricemia in subjects with this disease is still unknown Furthermore even if all factors responsible for hyperuricemia were known this knowledge would not in itself constitute a full explanation for the disease for it would not adequately explain the local tissue factors leading to precipitation of urate nor would it necessarily explain the mechanisms operating to initiate the acute attack of gouty arthritis in a person genetically endowed with the gouty trait

## CLINICAL FEATURES

A satisfactory definition of hyperuricemia is difficult to achieve As with virtually all constituents of serum the majority of uric acid values in normal subjects are grouped within a relatively small range The dimensions of the normal range must be defined either statistically or empirically since there exists no precise criterion for establishing an unequivocal upper limit of the normal range

### NORMAL URIC ACID VALUE IN SERUM OR PLASMA

Studies on the distribution of serum urate values in the general population must be interpreted with reference to the method employed In

6 0 mg per 100 ml in males as potentially abnormal Experience with the Praetorius method is less extensive but in the author s laboratory any value above 6 7 mg per 100 ml in males is considered potentially abnormal since in a comparison of the Folin and Praetorius methods on about fifty samples from normal and gouty subjects values by the latter method have averaged about 13 per cent higher

## SERUM URIC ACID VALUES IN GOUT

Using presently acceptable colorimetric methods the serum urate concentration in gout is almost invariably above 6 mg per 100 ml and may be as high as 15 mg per 100 ml though seldom higher In 177 serum analyses in 21 gouty patients published by Jacobson [11] the urate concentrations ranged from 5 2 to 14 8 mg per 100 ml 98 per cent exceeding 6 0 mg per 100 ml and 91 per cent exceeding 7 0 Talbott and Coombs [24] and Talbott [25] found concentrations ranging from 5 7 to 16 2 mg per 100 ml in over 900 determinations on 100 gouty patients Over 98 per cent of all values were above 6 0 mg per 100 ml Goldthwait Butler and Stillman [26] found values ranging from 4 8 to 16 0 mg per 100 ml in 113 patients with gout but in only 3 were the values below 6 mg per 100 ml and these were in patients with atypical gout

No large series of gouty patients has been studied with the Praetorius method but several patients with typical gout have had values in the range of 6 8 to 7 5 by this method [27 28] However these values may have been influenced by dietary factors for protein and purine restriction have been found to lower the serum urate level by 1 to 2 mg per 100 ml after a few days [29]

Data on the frequency of hyperuricemia among relatives of gouty subjects (cf Genetic Considerations near the end of the chapter) suggest that asymptomatic hyperuricemia is perhaps five times as common as clinical gout

## ACUTE GOUT

The most common complication of hyperuricemia is acute gouty arthritis Primary gout may occur at any age but it is predominantly a disease of adult males In large series of gouty patients only about 5 per cent are females Typically an attack occurs without warning in the midst of exuberant health in the mid forties or early fifties About 50 per cent of initial attacks involve the great toe (podagra) occasionally the initial attack may be bilateral [30] Ninety per cent of gouty patients experience attacks in the great toe some time during the course of their disease Next in order of frequency as sites of initial involvement are the instep ankle heel knee and wrist Any joint in the body may at times be involved Hench [31] has emphasized that the more distal the site of involvement the more typical is the character of the attack

TABLE 21 1 SERUM URIC ACID CONCENTRATIONS IN MILLIGRAMS PER 100 ML IN CONTROL SUBJECTS

| *Method* | *Males* | | | | *Females* | | | | *Reference* |
|---|---|---|---|---|---|---|---|---|---|
| | *No* | *Range* | *Mean* | *s d* | *No* | *Range* | *Mean* | *s d* | |
| Colorimetric (Folin) | 63 | 2 0-6 7 | 4 4 | 0 03 | 37 | 1 9-5 9 | 4 0 | 0 11 | [16] |
| Colorimetric (Benedict) | 206 | | 3 95 | 1 15 | 294 | | 3 63 | 1 06 | [1 ] |
| Colorimetric uricase (Buchanan) | 49 | | 5 4 | 1 2 | 53 | | 4 1 | 0 9 | [13] |
| Differential spectrophotometric (Praetorius) | 143 | 2 6-7 5† | 5 01 | 1 2 | 157 | 2 0-5 7† | 3 84 | 0 93 | [14] |
| Differential spectrophotometric (Praetorius) | 150 | 2 5-9 0 | 5 1 | 0 1 | 150 | 2 2-6 6 | 4 0 | 0 1 | [15] |

S I M

† Range = M ± 2 s d which included 97 per cent of observations in each sex

Fig 21 1 Chronic gouty arthritis of hands Note the extensive destruction of bone by urate deposits and the large soft tissue tophi

Fig 21 2 Chronic gouty arthritis of feet of same patient shown in Fig 21 1

It is difficult to improve on Sydenham's [32] description of the acute attack

> The victim goes to bed and sleeps in good health About two o clock in the morning he is awakened by a severe pain in the great toe more rarely in the heel ankle or instep This pain is like that of a dislocation and yet the parts feel as if cold water were poured over them Then follow chills and shivers and a little fever The pain which was at first moderate becomes more intense With its intensity the chills and shivers increase After a time this comes to its height accommodating itself to the bones and ligaments of the tarsus and metatarsus Now it is a violent stretching and tearing of the ligaments—now it is a gnawing pain and now a pressure and tightning So exquisite and lively meanwhile is the feeling of the part affected that it cannot bear the weight of the bedclothes nor the jar of a person walking in the room The night is passed in torture sleeplessness turning of the part affected and perpetual change of posture the tossing about of the body being as incessant as the pain of the tortured joint and being worse as the fit comes on Hence the vain effort by change of posture both in the body and the limb affected to obtain an abatement of the pain

The attack usually abates spontaneously in 1 to 2 weeks but may last several weeks Recovery following the initial episode is generally complete

## INTERVAL GOUT

The asymptomatic phase may last from a few weeks to many years Generally in 6 months to 2 years the patient will suffer another episode in the same or another joint and the events of the initial attack will be repeated unless modified by therapy In the natural history of the disease acute attacks recur with increasing frequency Eventually gouty arthritis with chronic involvement persists and joint deformity and destruction may occur these must not be considered simply a later stage of acute gouty arthritis since the two phases have different pathogeneses

## CHRONIC GOUTY ARTHRITIS

This is not merely a prolongation of an acute attack It may begin insidiously and progress with or without the superimposition of further attacks in the involved or other joints The duration of time from the initial attack to the beginning of chronic symptoms is highly variable ranging from 3 to 42 years with an average of 11 6 years [33] In one unique case widespread ankylosis developed following the initial gouty attack [34]

*Characteristic of the chronic stages of gouty arthritis* is the accumulation of urate in and around the affected joints In severe cases the accumulations may be enormous and the deformities of hands or feet grotesque (Figs 21 1 21 2)

The depositions of solid urate are not limited to joint regions nor are they dependent upon prior occurrence of articular gout Tophi form

Fig 21 1 Chronic gouty arthritis of hands Note the extensive destruction of bone by urate deposits and the large soft tissue tophi

Fig 21 2 Chronic gouty arthritis of feet of same patient shown in Fig 21 1

with some frequency in cartilaginous tissue of the body particularly the helix and antihelix of the ear Large accumulations may develop in the olecranon or patellar bursae Rarely, patients may show tophi at the time of the initial attack [35], before uricosuric agents came into general use about 50 per cent developed tophi at some time during the course of their disease [30] In three large series of gouty subjects recently reported [36–38] the incidence of tophi ranged from 13 to 25 per cent Tophi were found chiefly in patients with gout of 6 to 10 years duration or more

### RENAL IMPAIRMENT

With the passage of time the gouty patient tends to develop renal impairment as a complication of the disease The advent of renal insufficiency is a particularly serious matter for the gouty subject, for in its presence accumulation of urate is apt to progress at an accelerated pace After the age of 50 the incidence of tophi is significantly greater when renal damage is present than when it is absent [39]

It has long been appreciated that renal disease is an important cause of death in gout From 30 to 50 per cent of gouty patients are said to die of renal disease The most common indications of early renal damage are loss of ability to concentrate solids mild proteinuria and a reduction in excretion of phenolsulfonphthalein Marked proteinuria and retention of urea may occur later and are indicative of serious renal involvement and a poor prognosis [39]

## PATHOLOGY

The specific pathologic changes in gout depend upon the deposition of urate in tissues and the associated inflammatory reactions and degenerative changes The one pathognomonic lesion of gout is the tophus a urate deposit surrounded by tissue exhibiting inflammatory and foreign body reaction Because urate crystals are water soluble nonaqueous fixatives are necessary to preserve the urate deposits in histologic sections Urate crystals if preserved in bulk are brilliantly anisotropic when viewed with polarized light under the microscope A useful and simple staining technique is that of de Galantha in which tissue is fixed in absolute alcohol and subsequently stained with silver [40] The urate crystals are stained brown black by this method However provided the initial fixation is done with absolute alcohol staining with hematoxylin and eosin is also usually successful even though the bulk of the crystals may have been dissolved out

Two general categories of mechanisms have been proposed regarding deposition of urate in gout Borrowing some terms from concepts of pathologic calcification Sokoloff [41] has grouped these as follows (1) *metastatic*, implying that urate is deposited in tissues because excessive quantities are presented to them by circulating blood (2) *dystrophic*

implying that urate is deposited in tissues because the latter have undergone some primary pathologic alteration rendering them susceptible of urate deposition These two categories are not mutually exclusive

## THE TOPHUS

The characteristic tophaceous nodule consists of the multicentric deposit of urate crystals and intercrystalline matrix together with the inflammatory reaction and foreign body granuloma it has evoked (Fig

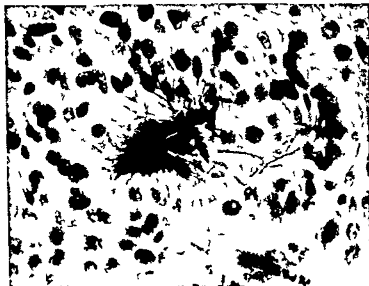


Fig 21-3 Acute gouty synovitis The tissue has been fixed in absolute alcohol and the section stained by the method of de Galantha Individual and aggregated needle-like crystals have deposited in incipient tophus formation This lesion has been included in The Pathology Teaching Collection of Lantern Slides (*By permission of the Arthritis and Rheumatism Foundation and of Leon Sokoloff*)

21-3) [41] The crystals generally believed to be monosodium acid urate monohydrate [42] are acicular and arranged radially in small clusters Granular and amorphous deposits have also been described in articular accumulation Calcific material deposited in the intercrystalline matrix may render the tophus radiopaque This process though uncommon may at times reach the proportions of heterotopic ossification [43] A variety of components protein lipid and polysaccharide in nature has been described in the gouty tophus Some workers view the urate deposits as occurring secondary to tissue changes involving such materials The term *tophus* is occasionally reserved for urate deposits in tissue other than joints although this distinction has no pathologic basis Tophi are commonly seen in the helix or antihelix of the ear the olecranon and patellar bursae and tendons Less frequently they occur in the skin of palm

or soles the tarsal plates of the eyelids nasal cartilages finger tips and in the cornea and sclerotic coats of the eye Rarely they have been observed in the corpus cavernosum and prepuce of the penis, the aorta myocardium aortic or mitral valves the tongue, epiglottis, vocal cords and arytenoid cartilages [30]

It has been pointed out that urates tend to deposit in relatively avascular tissue [30] Tissue alterations of unknown type including evolution of substances in the intercrystalline matrix may play a role in precipitating or maintaining the tophus Such alterations possibly the result of previous injury, might be expected to occur more readily in tissues with a limited blood supply or in tissues such as cartilage which depend on perfusion by body fluids for nutritional requirements Cartilage has an affinity for absorbing urates in vitro [44] and tarsal bones of swine will cause precipitation of needles such as seen in gout when suspended in saturated solutions of urate [45] Sokoloff [41] has pointed out that several sites of urate deposition in gout—articular and other cartilages synovial tissues interstitial tissue of the renal pyramid and sclerae and heart valves—have in common ground substance containing acid mucopolysaccharide If a local pH change is of importance in initiating precipitation of urate from a saturated solution which at pH 7 4 would be comprised of 98 9 per cent monosodium urate and 1 1 per cent uric acid then one might expect that the crystals would actually be uric acid whose solubility is remarkably le s than that of its monosodium salt Indeed on crystallographic evidence de Graciansky concluded that the gouty crystalline deposits were probably uric acid rather than the salt [46] This important problem warrants reinvestigation

## ACUTE GOUTY ARTHRITIS

The mechanism precipitating the acute gouty attack is unknown as is the role of uric acid in this phenomenon A few leads have emerged on the metabolic front in recent years and these will be discussed below Suffice it to say here that many workers [13 47] entertain serious doubts that uric acid itself is immediately responsible for the local and systemic disturbances in acute gouty arthritis

A question of paramount importance therefore concerns whether urate is deposited in synovial tissues at the time of or immediately prior to the first attack Recently Zevely and coworkers [48] found urate crystals in four of six instances in which tissue was obtained by punch biopsy Sokoloff [41] also has reported the occurrence of urate crystals in the synovium during the acute attack There was associated acute synovitis and in Sokoloff s case a submiliary granuloma but no fibrosis These observations were interpreted as evidence that urates are deposited in the articular tissues *during* acute gouty episodes However these patients had had prior attacks and the possibility of earlier deposition

is difficult to exclude It has also been known for many years that urate depo its may develop in joints in idiou ly and may not give ri e to acute symptoms [49 50]

Villa Robecchi and Ballabio [51] obtained a biopsy specimen of synovial membrane from a patient suffering from a first attack of gout There was acute exudative inflammation without urate deposits Such negative evidence is of course inconclusive since it offers no information regarding possible crystals el ewhere in the synovium It is at present uncertain whether the nonspecific acute inflammatory reaction of acute gout is in re ponse to or accompanied by the depo ition of urate crystals

## CHRONIC GOUTY ARTHRITIS

Urate crystals deposit in the synovial membrane articular cartilage subchondral bone capsular and periarticular ti sues tendons and at times the overlying skin The crystals are deposited radially in the super ficial portions of the articular cartilage This is generally interpreted as evidence that urates enter the cartilage from the synovial fluid [52]

Synovial involvement becomes more pronounced as the disease ad vances A variable degree of nonspecific acute and chronic inflammatory reaction accompanies the tophaceous reaction in the synovial membrane

The affected portions of the articular cartilage undergo nonspecific degenerative changes Sokoloff [41] has distinguished three patterns of joint involvement that may occur as a consequence of cry tal deposits (1) disintegration of the articular cartilage may lead to o teoarthritis (2) urate depo its may cause ma ive obliteration of joint structures (3) chronic tophaceous synovitis with the associated inflammatory reaction may lead to mall areas of pannus formation rarely rheumatoid like ankylo is may occur

No joint is exempt although joints of the lower extremity are mo t commonly involved In vertebral bodie urate depo its are found in marrow spaces adjacent to intervertebral di ks as well as in disk tis ue itself The punched-out le ions of bone commonly seen in roentgenograms (Fig 21 1) of gouty patients represent marrow tophus depo its which in mo t in tances communicate with the urate cru t on the articular urface through ero ions and defect in the articular cartilage (Fig 21-4) [41] The urate depo its of articular cartilage though at times exten ive lack the inflammatory and foreign body reaction characteri tic of the tophus

Crystals have been recovered from the putum of gouty subjects but they apparently do not occur in the central nervous ystem

## THE KIDNEY IN GOUT

The kidneys of most patient with gout contain depo its of urate in the medulla [53] The term *gout nephrosis* has been applied to the micro-

tophaceous urate deposits in the renal pyramids [41] Minkowski [54] believed that they were first deposited in the epithelium of the convoluted tubules and were subsequently flushed into the collecting tubules where an inflammatory reaction destroyed the tubular structure Urate crystals occur frequently in the interstices of the pyramid Here they evoke an inflammatory reaction and may lead to foci of necrosis [55]



Fig 21-4 Chronic gouty arthritis Urate crystals in the synovial membrane articular cartilages and subchondral bone appear black Advanced osteoarthritic changes with marginal osteophyte formation are seen (*From L Sokoloff and I O Gleason Am J Clin Path* 24 406 1954 *with permission of authors and publisher*)

In a small proportion of cases the pyramidal deposits of urate are so great as to cause serious mechanical obstruction of the nephrons This results in atrophy and fibrosis of the tubule with variable secondary chronic inflammatory changes The end result may be a scarred kidney variously interpreted as obstructive nephropathy or chronic pyelonephritis Modern and Meister [56] have drawn attention to the combination of progressively increasing azotemia decreased phenolsulfonphthalein excretion and a fixed specific gravity of the urine in gouty patients in whom hypertension albuminuria and cylinduria are absent They believe these findings characteristic of the tophaceous kidney of gout

Evidence for gout nephrosis was cited by Sokoloff [41] in 64 of 80 cases of gout coming to autopsy but in only 5 cases were the changes considered severe Arteriolar sclerosis and nephrosclerosis were also commonly seen

Since renal disease is common in those with recognized gout one might

anticipate that instances of renal disease should also occur in persons possessing the gouty trait but not manifesting typical clinical gout Two distinct types of primary renal involvement are worthy of comment

The first of these is nephrolithiasis The stones are composed of uric acid not biurate [56a] Calculi are said to occur in 12 to 15 per cent of clinically gouty patients [39 57] and are particularly common during uricosuric therapy However it is also known that calculi occur with some frequency among hyperuricemic subjects who do not have clinical gout For example Boyce (quoted by Talbott [39]) reports an incidence *of hyperuricemia of 22 per cent but of gout of only 10 per cent among* patients passing pure uric acid stones

The second type of involvement first described by Ebstein [55] is that of gout nephrosis occurring without clinical evidence of gout Umber [58] also described two cases of typical gouty kidneys with no clinical history or other pathologic findings of gout Brown and Mallory [53] have described one patient who probably falls into this group In none of these cases were uric acid analyses of blood or serum performed during life

It is likely that renal disease secondary to clinically occult gout is more common than presently recognized [1] It should be suspected whenever hyperuricemia seems out of proportion to the degree of renal impairment in patients in whom an adequate cause for the renal disease is not apparent The suspicion would be enhanced by finding asymptomatic hyperuricemia or clinical gout among family members A positive diagnosis *during life is difficult to achieve however since translumbar renal* biopsy does not yield the medullary tissue necessary for adequate pathologic study

### VASCULAR SCLEROSIS IN GOUT

Arteriosclerosis especially in the kidney and hypertension are said to have an increased incidence in gout particularly in patients who have had their disease for 15 to 20 years [59] Renal functional impairment is probably attributable to nephrosclerosis in most cases rather than to gout nephrosis [60] Renal involvement tends to be only slowly progressive [39] and life expectancy and mortality are reportedly normal in gout [61]

## BIOCHEMISTRY OF PURINE COMPOUNDS

### BIOSYNTHESIS OF THE PURINE RING

Early work concerned with the biosynthesis of purine compounds in animals and man has been reviewed elsewhere [62-65]

[1] A clear distinction should be made between this concept and the point of view that renal insufficiency per se may be a cause of gout as well as of hyperuricemia Evidence for the latter view is meager and inconclusive Some workers have suggested that latent gout may be activated by renal insufficiency These topics are discussed more fully elsewhere [38a].

For many years there was considerable uncertainty regarding the synthesis of the purine ring Because of structural similarities urea [66] arginine and histidine [67] were proposed as precursors of purines When experiments were performed with these substances in labeled form [68, 69] and no labeling of tissue purines resulted, these theories were discarded In 1943 Barnes and Schoenheimer [68] fed ammonium citrate containing $N^{15}$ to pigeons and demonstrated that the purines of internal organs and the uric acid of the excreta contained appreciable $N^{15}$ in the ring structure and substituent amino groups Analogous experiments in rats yielded qualitatively similar data These pioneer experiments demonstrated that the purine ring was synthesized within the body from small molecules

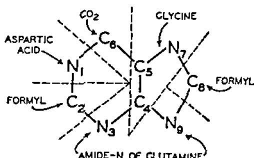



Fig 21-5 Origins of the atoms of the purine ring (*From Metabolism* 6 244 1957 *with permission of the publisher*)

Since these early studies rapid strides have been made The use of various labeled substrates in bacterial avian and mammalian systems has clearly defined the origin of the individual atoms of the purine ring More recently work with soluble enzyme systems has delineated the biosynthetic sequence in a stepwise manner so that today the metabolic origin of the major purine compounds is well known

In Fig 21 5 are shown the precursors of the various carbon and nitrogen atoms in the purine ring The basic building block is the simplest of amino acids glycine which contributes carbon atoms 4 and 5, and nitrogen atom 7 [70 72] Carbon atoms 2 and 8 come from formate [72 73] carbon atom 6 comes from $CO_2$ [71 74] nitrogen atoms 3 and 9 come from the amide-N of glutamine [75–77] and nitrogen atom 1 comes from aspartic acid [76] Compounds which are converted to glycine in the body will also contribute to the 4 5 and 7 positions of uric acid Similarly compounds which donate one-carbon fragments to the "formyl" pool, will contribute to carbons 2 and 8 of purines

The biosynthesis of purine compounds may logically be considered to begin with the formation of an active ribose derivative α 5-phosphoribosyl 1 pyrophosphate abbreviated PP ribose-P (Fig 21-6) This

compound is formed by a transfer of the terminal pyrophosphate group of ATP to the first carbon of ribose 5 phosphate [78 79]



Ribose 5 phosphate + ATP ⟶ PP ribose P + AMP (1)

Ribose 5-phosphate has three possible pathways of origin it may arise as an intermediate in the 6-phosphogluconic acid oxidation pathway of glucose as a product of nonoxidative cleavage of fructose 6-phosphate or as a late product of the metabolism of glucose 1 phosphate via the uronic acid cycle The 6-phosphogluconic acid pathway is probably the major source of ribose esters (See Chap 4)

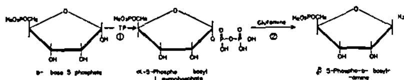
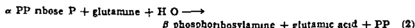



Fig 21 6 Biosynthesis of phosphoribosylamine The encircled numbers in this and subsequent figures refer to numbered reactions in the text

PP ribose-P is involved in purine synthesis in two types of reactions One is formation of the first specific intermediate of purine synthesis *de novo* 5-$\beta$-phosphoribosyl 1 amine [80–82] and the other is direct synthesis of ribonucleotides from the free purine base by a pyrophosphorylase mechanism

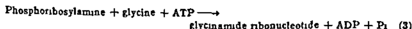
In the former reaction the pyrophosphate group of PP ribose-P is displaced by the amide group of glutamine and there is an inversion of the substituent to yield the $\beta$ linkage characteristic of the glycosidic bond of all known ribonucleotides [82]



$\alpha$ PP ribose P + glutamine + $H_2O$ ⟶
$\beta$ phosphoribosylamine + glutamic acid + PP (2)

The exact mechanism of this reaction is not yet understood but both PP ribose-P and glutamine are bound independently by the enzyme prior to the reaction [83] The reaction generating phosphoribosylamine is the first irreversible reaction dealing specifically with synthesis of purines *de novo* and it thus becomes a potentially important reaction from the standpoint of control of purine synthesis

The stepwise synthesis of the purine ring is shown in Fig 21 7 Phosphoribosylamine reacts with glycine to yield glycinamide ribonucleotide ATP is involved in this reaction as an energy source [84–86]



Phosphoribosylamine + glycine + ATP ⟶
glycinamide ribonucleotide + ADP + $P_i$ (3)

For many years there was considerable uncertainty regarding the synthesis of the purine ring Because of structural similarities urea [66] arginine, and histidine [67] were proposed as precursors of purines When experiments were performed with these substances in labeled form [68 69] and no labeling of tissue purines resulted these theories were discarded In 1943, Barnes and Schoenheimer [68] fed ammonium citrate containing $N^{15}$ to pigeons and demonstrated that the purines of internal organs and the uric acid of the excreta contained appreciable $N^{15}$ in the ring structure and substituent amino groups Analogous experiments in rats yielded qualitatively similar data These pioneer experiments demonstrated that the purine ring was synthesized within the body from small molecules

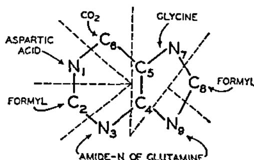


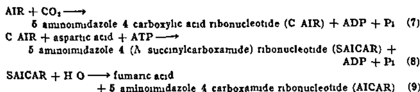

Fig 21 5 Origins of the atoms of the purine ring (*From Metabolism* 6 244 1957 *with permission of the publisher*)

Since these early studies rapid strides have been made The use of various labeled substrates in bacterial avian and mammalian systems has clearly defined the origin of the individual atoms of the purine ring More recently work with soluble enzyme systems has delineated the biosynthetic sequence in a stepwise manner so that today the metabolic origin of the major purine compounds is well known

In Fig 21-5 are shown the precursors of the various carbon and nitrogen atoms in the purine ring The basic building block is the simplest of amino acids glycine which contributes carbon atoms 4 and 5, and nitrogen atom 7 [70 72] Carbon atoms 2 and 8 come from formate [72 73] carbon atom 6 comes from $CO_2$ [71 74] nitrogen atoms 3 and 9 come from the amide N of glutamine [75–77] and nitrogen atom 1 comes from aspartic acid [76] Compounds which are converted to glycine in the body will also contribute to the 4 5 and 7 positions of uric acid Similarly, compounds which donate one-carbon fragments to the 'formyl' pool, will contribute to carbons 2 and 8 of purines

The biosynthesis of purine compounds may logically be considered to begin with the formation of an active ribose derivative $\alpha$ 5-phosphoribosyl 1 pyrophosphate abbreviated PP ribose-P (Fig 21-6) This

5-Aminoimidazole ribonucleotide (AIR) now receives a carboxyl group at $C_4$ by a $CO_2$-fixation reaction [92] which probably involves biotin as a coenzyme [93] The carboxyl serves as a point for condensation of this intermediate with aspartic acid through an amide linkage involving another ATP as the source of energy [94] Hydrolysis of the intermediate now yields 5 aminoimidazole-4 carboxamide ribonucleotide a compound lacking only the number 2 carbon atom of a complete purine ribonucleotide

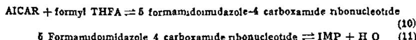


AIR + $CO_2$ ⟶
5 aminoimidazole 4 carboxylic acid ribonucleotide (C AIR) + ADP + Pi (7)

C AIR + aspartic acid + ATP ⟶
5 aminoimidazole 4 (N succinylcarboxamide) ribonucleotide (SAICAR) + ADP + Pi (8)

SAICAR + $H_2O$ ⟶ fumaric acid
+ 5 aminoimidazole 4 carboxamide ribonucleotide (AICAR) (9)

AIC-ribonucleotide receives a second formyl group from a tetrahydrofolic acid derivative [95 96] and ring closure completes the biosynthesis of the purine structure by forming hypoxanthine ribonucleotide or inosine 5 monophosphate (IMP) as it is usually termed [96]



AICAR + formyl THFA ⇌ 5 formamidoimidazole-4 carboxamide ribonucleotide (10)

5 Formamidoimidazole 4 carboxamide ribonucleotide ⇌ IMP + $H_2O$ (11)

One intermediate in the synthesis of IMP deserves special comment viz 5-aminoimidazole-4-carboxamide ribonucleotide Its free base 4(5) aminoimidazole 5(4) carboxamide (AIC) was first isolated in 1945 by Stetten and Fox [97] from cultures of *Escherichia coli* grown in the presence of sulfonamide The elucidation of its structure by Shive and coworkers [98] stimulated considerable interest since it was immediately recognized as a potential purine precursor This postulate was confirmed when Miller Curtis and Wilson [99] demonstrated incorporation of AIC-$C^{14}$ into purines and purine catabolites in the rat Other studies further substantiated the utilization of AIC for purine synthesis [100] It eventually became clear however that it was not free AIC but rather its ribonucleotide which was the direct precursor of IMP [101 102] Labeled AIC has proved to be an extremely useful compound in studies of purine synthesis in gouty subjects as discussed below

## BIOSYNTHESIS OF OTHER NUCLEOTIDES

IMP may be considered the parent purine compound It has several possible fates (Fig 21 8) First to be considered here is its role as an intermediate in the formation of adenosine 5 monophosphate (AMP) and

Thus at this early stage the fundamental components of the eventual polynucleotide structure of nucleic acids are already joined and a combination of base, sugar, and phosphoric acid has been made

Fig 21 7 Biosynthesis of the purine ring

Glycinamide ribonucleotide receives a one-carbon formyl unit from an active folic acid derivative [84 85] possibly $N^5$, $N^{10}$ anhydroformyl tetrahydrofolic acid (THFA) [87 88]

Glycinamide ribonucleotide + $N^5$ $N^{10}$ anhydroformyl THFA ⟶
α N formylglycinamide ribonucleotide (FGAR) + THFA (4)

The formyl derivative then receives the amide group of glutamine to form the corresponding amidine compound [89 90] following which ring closure occurs to form the five membered imidazole ring [91] one of two fused heterocyclic rings comprising the eventual purine structure Each of these reactions requires ATP as an energy source

FGAR + glutamine + ATP ⟶
α N formylglycinamidine ribonucleotide (FGAM) + glutamic acid + ADP + $P_i$ (5)

FGAM + ATP ⟶ 5 aminoimidazole ribonucleotide + $H_2O$ + ADP + $P_i$ (6)

AMP and GMP may be converted to di and triphosphates which are essential coenzymes of many reactions and building blocks for nucleic acids

### RECONSTITUTIVE REACTIONS

Mechanisms exist for resynthesis of ribonucleotides from free bases or ribonucleosides both of which must have arisen from prior cleavage of nucleotides synthesized *de novo* by pathways just discussed

Free purine bases may be converted to ribonucleotide form by reacting directly with PP ribose-P according to the following scheme



Base + PP ribose P ⇌ base ribose phosphate + PP (16)

Two different purine ribonucleotide pyrophosphorylases have been identified one acting on AIC and adenine [*112*] another on hypoxanthine guanine and 6 mercaptopurine [*113* *114*] These enzymes are widely distributed and have been studied most extensively in yeast [*102*] beef liver [*112* *113*] and human erythrocytes [*114*]

A second mechanism involves conversion of base to ribonucleoside by reversal of the nucleoside phosphorylase reaction [*115–117*] and subsequent conversion of ribonucleoside to ribonucleotide by action of the appropriate kinase [*118*, *119*] according to the following schemes



Base + ribose 1 phosphate ⇌ base ribose + Pi (17)



Base ribose + ATP ⟶ base ribose phosphate + ADP (18)

Nucleoside phosphorylases are widely distributed in mammalian tissue and are particularly active in liver [*117*] and erythrocytes [*120*] At least in the case of inosine the equilibrium point is far toward the ribonucleoside [*116*] Nucleoside kinase reactions have been studied less extensively in mammalian systems but do appear to exist For example Goldthwait [*121*] found adenosine to be converted to acid soluble nucleotides by dog heart muscle even more actively than was adenine Similarly Lowy et al [*122*] have shown that AIC ribonucleoside is readily utilized by rabbit erythrocytes for synthesis of ATP and GTP

Reutilization of purine bases and nucleosides released by catabolism of nucleic acids and coenzyme nucleotides has been viewed by Kornberg [*123*] as a salvage process By either of these reconstitutive pathways only one high-energy bond in the form of PP ribose P or ATP is expended in the synthesis of a nucleotide whereas in the synthesis of IMP *de novo* from glycine and PP ribose P a minimum of four ATP molecules is required

In addition to the salvage function performed by these reactions it is probable that they are also important primary biosynthetic mechanisms in some tissues [*124*] which are dependent upon a supply of preformed

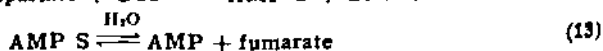
guanosine 5 monophosphate (GMP), the purine nucleotide components of nucleic acids IMP is aminated in the 6 position to form AMP [103] This conversion occurs in two steps involving an initial condensation of IMP with aspartic acid to form adenylosuccinic acid (AMP S) [104 105] The energy necessary for this reaction is derived from the cleavage of

Fig 21 8 Biosynthesis of purine ribonucleotides ribonucleosides and bases

guanosine triphosphate [106] but the reaction is complex and its mechanism poorly understood [106 107] Hydrolysis of AMP-S yields AMP and fumaric acid This latter reaction is freely reversible [106]

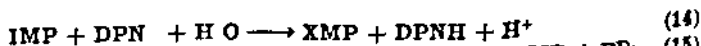


$$\text{IMP} + \text{aspartate} + \text{GTP} \longrightarrow \text{AMP S} + \text{GDP} + \text{P}_i \quad (12)$$

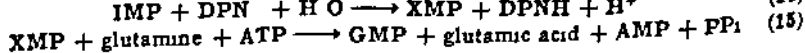


$$\text{AMP S} \overset{\text{H}_2\text{O}}{\rightleftharpoons} \text{AMP} + \text{fumarate} \quad (13)$$

The conversion of IMP to GMP also occurs in two steps The first is irreversible oxidation of IMP to xanthosine 5 monophosphate (XMP) with diphosphopyridine nucleotide (DPN) as hydrogen acceptor [108 109] The second is the amination of XMP at position 2 and the specific amino donor for the reaction is the amide group of glutamine [110 111] This step requires ATP as the source of energy These reactions proceed according to the over all schemes shown below



$$\text{IMP} + \text{DPN} + \text{H}_2\text{O} \longrightarrow \text{XMP} + \text{DPNH} + \text{H}^+ \quad (14)$$



$$\text{XMP} + \text{glutamine} + \text{ATP} \longrightarrow \text{GMP} + \text{glutamic acid} + \text{AMP} + \text{PP}_i \quad (15)$$

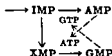
fraction of pigeon liver reduced synthesis of IMI from glycine by 90 per cent [126]

CONTROL OF SALVAGE PATHWAYS

Way and Parks [131] have shown that the conversion of certain purine analogues to their respective ribonucleotides by the pyrophosphorylase reaction (Reaction 16) is also strongly inhibited by ADP or ATP These results suggest the existence of another negative feed back control suppressing the formation of new nucleotides from purine bases when adequate concentrations of nucleotides are already present

CONTROL OF RIBONUCLEOTIDE INTERCONVERSION

The cofactor in the conversion of IMP to AMP is GTP whereas the cofactor in the conversion of XMP to GMP is ATP Stetten [132] has suggested that the reciprocal roles of the triphosphates in the formation of the monophosphates may help preserve a balance between AMP and GMP synthesis by a positive feed back control




In a bacterial system studied by Magasanik the conversion of IMP to XMP is inhibited by CMP or other guanine compounds [133] A potential feed back regulation of GMP synthesis from IMP is thus disclosed 6 Mercaptopurine ribonucleotide has also been shown to inhibit conversion of IMP to XMI as well as conversion of IMP to AMP [134] This latter conversion is also inhibited by 2 6-diaminopurine or derivatives thereof [135] These results suggest the possible existence of a control of AMP synthesis from IMP by natural ribonucleotides but this awaits demonstration In a bacterial system the synthesis of AMI from IMP is regulated in a novel way In the sequence IMP ⟶ XMP ⟶ GMP ⟶ IMI the terminal reaction is strongly inhibited by ATP [133] This cycle is not known to occur in mammalian systems

The feed back controls presently demonstrated as potentially operating on nucleotide interconversions are shown in Fig 21 9

In pigeons and other uricotelic organisms the unrestrained synthesis of IMI is the major process for disposal of waste nitrogen and results in generation of large quantities of hypoxanthine and ultimately of uric acid rather than overproduction of nucleic acids Evidence to be presented below suggests that an appreciable amount of uric acid is also synthesized in normal man by such a shunt pathway and that abnormal amounts may be made in certain gouty subjects by this or other routes which by pass nucleic acids The mechanism whereby IMP is

purine precursors for their nucleic acid synthesis Lajtha and Vane [125] have demonstrated that bone marrow, for example is dependent upon a preformed purine precursor originating in liver for its source of nucleic acid purine bases The exact form of the precursor is not identified

## NORMAL CONTROL MECHANISMS

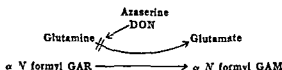
### REGULATION OF PURINE SYNTHESIS DE NOVO

The first *irreversible* reaction concerned *specifically* with purine synthesis is that in which β phosphoribo ylamine is generated from α phosphoribosylpyrophosphate and glutamine [80 82] by the enzyme named phosphoribosylpyrophosphate amidotransferase [82] Pho phoribo yl amine has no known function other than its role in purine synthe is *de novo* It is however, an unstable compound and is readily broken down if not promptly utilized Because of these factors the rate-limiting step in purine biosynthesis may be the reaction generating phosphoribosyl amine [83 126]

The rate of generation of phosphoribosylamine is in turn dependent upon the activity of the amidotransferase and the abundance of glutamine and PP ribose P Activity of the amidotransferase is influenced by certain purine ribonucleotides which are competitive inhibitors of binding of PP ribose-P but not of glutamine by the enzyme ADP and ATP are the most potent of the inhibitory ribonucleotides and the dissociation constants of their enzyme-inhibitor complexes are some six times the $K_m$ of PP ribose-P [83] A potential feed back control of purine biosynthe is *de novo* by purine ribonucleotides is thus disclosed since ADP and ATP are present in cells in appreciable concentration [127 128] and PP ribo e-P is probably present in relatively low concentration No natural factors which compete with glutamine for binding by the enzyme are known and it is unlikely that glutamine is involved in a regulatory mechanism The effects of high protein diets in augmenting purine synthesis [139] may operate through glutamine however since its $K_m$ in this reaction is quite high $1\ 1 \times 10^{-3} M$ (pigeon liver enzyme)

*Factors tending to lower concentrations of substrates may* also reduce phosphoribosylamine synthesis and thus reduce purine synthe is *de novo* Some factors have been studied with re pect to PP ribose-P Since this ester is also utilized in reactions which generate ribonucleotides from purine and pyrimidine ba es directly the availability of PP ribose-P for purine synthesis *de novo* will be influenced by the balance achieved between rates of synthesis of PP ribose P and of utilization in variou ribonucleotide pyrophosphorylase reactions The inhibition of purine synthesis *de novo* by preformed purine bases [126 130] may represent chiefly diversion of PP ribose P from the *de novo* sequence For example inclusion of adenine ($2 \times 10^{-3} M$) in an incubation mixture of the soluble

equence Although these substances act as glutamine antagonists in each of the three reactions of purine synthesis in which glutamine is a substrate [81 111 136] the step involving conversion of α-N formyl glycinamideribonucleotide to the corresponding amidine compound is by far the most sensitive to these agents [111 136]

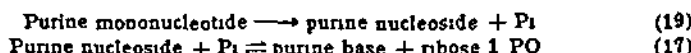



Azaserine [137] and DON [138] also inhibit glycine incorporation into uric acid in man presumably at this reaction site

## NUCLEIC ACID CATABOLISM

Enzymatic hydrolysis of the polynucleotide chains of nucleic acids occurs through the action of various nucleases [139] The major products released by ribonuclease a and b and by deoxyribonuclease I and II are oligonucleotides The oligonucleotides are further cleaved by phosphodiesterases to yield 5 - and 3 mononucleotides

The mononucleotides are split by group specific nucleo ide 5 phosphatases [140] as well as by a variety of nonspecific phosphatases [141] to yield the corresponding purine or pyrimidine nucleoside and orthophosphate The purine nucleoside is then split by purine nucleoside phosphorylase [115] to yield the free purine base and ribose or deoxyribose 1 phosphate [115 116]



Purine mononucleotide ⟶ purine nucleoside + Pi (19)
Purine nucleoside + Pi ⇌ purine base + ribose 1 PO (17)

Analogous reactions exist for pyrimidine nucleosides but since there is presently no evidence that pyrimidine compounds play a role in gout they will not be con idered in this chapter

In addition to these general reactions AMP and adenosine are acted upon by pecific deaminating enzymes AMP is converted to IMP by adenylic deaminase (Reaction 20) [142] and adenosine to inosine by adenosine deaminase (Reaction 21) [143] (Fig 21-8 )

### SUBSTITUTED PURINES

Recently certain other bases have been shown to exist as minor con tituents of nucleic acids The mononucleotides derived from DNA contain small quantities of 5 methylcytosine 5-hydroxymethylcytosine and 6-methylaminopurine in addition to adenine guanine thymine and cytosine [144] Those derived from RNA contain small quantities of thy

shunted into cleavage fates away from additional synthesis of AMP and GMP may involve feed back regulation of the further synthetic reactions as suggested above

The possibility also exists that conversions of IMP to AMP and XMP are already proceeding at near maximal rates because of enzyme saturations at low substrate concentrations of IMP Concentrations of IMP in excess of those capable of influencing further ribonucleotide synthesis might then result in increased production of hypoxanthine especially if the $K_m$ value of the nucleotide 5 phosphatase which initiates the

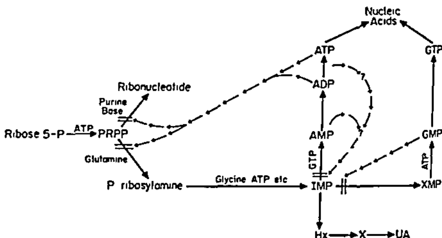



Fig 21 9 Potential feed back control of purine biosynthesis

cleavage sequence were rather high No $K_m$ data are available in avian or mammalian systems from which to evaluate this point

## OTHER FACTORS

It seems unlikely that any of the substrates which serve as specific donors of atoms of the purine ring would be limiting with respect to purine synthesis since they are present in abundance within the body and the fraction of their total turnover ultimately employed in purine synthesis must be small indeed Similarly it is unlikely that specific cofactors would be limiting Finally the activities of the various enzymes of the biosynthetic sequence eventually converting phosphoribosylamine and glycine to IMP do not appear to be limiting for the intermediates of this sequence normally do not accumulate and the corresponding ribonucleosides or aglycones do not appear in urine

No evidence was secured for any normal regulatory mechanism operating between glycinamideribonucleotide and IMP in pigeon liver extracts [126] The antibiotics azaserine and 6-diazo 5 oxo-L-norleucine (DON) do, however, inhibit purine biosynthesis at a site in this portion of the

oxidized by the same enzyme to yield uric acid (Reaction 24) [*152*] (Chap 22) It will be noted from Fig 21 8 that whereas adenine hypoxanthine and guanine appear to be derived exclusively by cleavage of the corresponding nucleoside xanthine has at least three direct precursors namely its nucleoside (xanthosine or deoxyxanthosine) free hypoxanthine and guanine

Until recently the only established reaction generating uric acid was the oxidation of xanthine A second potential pathway, involving phosphorolytic cleavage of uric acid riboside to uric acid and ribose 1 phosphate has now been demonstrated [*153*] The phosphorylase capable of catalyzing this cleavage is different from the general purine nucleoside phosphorylase and is specific for uric acid riboside among purine compounds but it will also cleave thymidine This finding raises a question as to the identity of its natural substrate Also unsettled is whether this enzyme is different from the thymidine phosphorylase discovered in kidney by Friedkin and Roberts [*154*] The occurrence of uric acid riboside is well established only in beef erythrocytes [*155*] and liver [*156*] Several workers have failed to find the compound in human erythrocytes [*157, 158*] though it was originally claimed to be there [*159*] The mode of biosynthesis of this compound is unknown and its significance is at present uncertain Nevertheless it constitutes a potential precursor of uric acid of considerable interest

## PURINES IN BIOLOGIC FLUIDS

### PLASMA

The predominant purine of plasma is uric acid The $pK_A$ and $pK_{A_2}$ of uric acid are 5 75 and 10 2 respectively [*160*] Therefore at pH 7 4 about 98 per cent of uric acid will exist as the monoalkali salt [*161*] assuming no complex formation and no physical binding by large molecules There is little evidence in man for aggregation of uric acid either with itself or with large molecules Dialysis equilibration studies have shown that less than 4 per cent of uric acid is bound to nondiffusible elements at plasma levels ranging from 2 to 7 mg per 100 ml [*163*] Ultrafiltration studies have shown that virtually all plasma urate of normal and gouty subjects is readily ultrafiltrable [*163*] In electrophoresis of plasma neither the natural uric acid originally present in plasma nor that added to it migrates with protein in both cases uric acid moves anodally ahead of albumin [*164*] These results have thus failed to confirm the presence of a uric acid–protein complex in blood [*165 166*]

Mono sodium urate is soluble in distilled water to the extent of approximately 100 mg per 100 ml [*167*] Its solubility in serum is considerably less probably because of effects of other ions present [*168*] Solubility of uric acid in serum in vitro has been reported as high as 25 mg per 100 ml

mine 2 methyladenine 6 methylaminopurine 6 dimethylaminopurine $N^2$ methylguanine (6-hydroxy 2 methylaminopurine) and 1 methyl guanine [145, 146] in addition to adenine guanine uracil and cytosine Thus far none of these new bases has been found in mammalian nucleic acids except for 6 methylaminopurine, which occurs in rabbit and rat liver [146] However since certain of them are found in human urine in small quantities the possibility exists that they may also occur in nucleic acids of man

## CATABOLISM OF INGESTED NUCLEOPROTEINS

Nucleic acids of dietary nucleoproteins are liberated in the intestinal canal by the action of proteolytic enzymes Nucleic acids are degraded in turn to nucleotides by nucleases and phosphodiesterases secreted by the pancreas The nucleotides are chiefly hydrolyzed to nucleosides by various nucleotidases and phosphatases and the nucleosides may be absorbed intact or they may be cleaved phosphorolytically to yield the free base The small intestinal mucosa of man is rich in nucleoside phosphorylase and xanthine oxidase [147] so ingested nucleoprotein purines may potentially be converted to uric acid prior to entrance into the blood stream From experiments in which normal and gouty subjects ingested $N^{15}$ labeled nucleic acids [148] it appeared that the purine moieties were converted to uric acid largely by direct routes without prior incorporation into body nucleic acids These results accord with the information presented above and also with the observation that persons placed on purine free diets show a prompt fall in urinary uric acid excretion reaching stable low values in 2 to 4 days That small quantities of dietary nucleosides and even nucleotides may be utilized directly for synthesis of nucleic acids and other metabolic functions in the body is also suggested by recent work [149]

# FORMATION OF URIC ACID

The free purine bases that result from nucleoside cleavage are adenine guanine hypoxanthine and xanthine Since purine nucleoside phosphorylase acts most readily upon inosine and guanosine [115 117 150] the major bases generated are very likely hypoxanthine and guanine In mammalian tissue free adenine is not deaminated as it is in certain *bacterial systems* [141] so that adenine does not give rise directly to hypoxanthine If it is not reconverted to its nucleoside or nucleotide it may be excreted unchanged and normal human subjects excrete adenine *in small quantities in urine* [151] *In contrast* the other purine bases are readily converted to uric acid Guanine is deaminated by guanase to yield xanthine (Reaction 22) Hypoxanthine is oxidized by xanthine oxidase to yield xanthine (Reaction 23) which in turn is further

Uric acid riboside exists in beef erythrocytes but modern chromatographic methods have failed to confirm its presence in human erythrocytes

Uric acid itself is present in erythrocytes but its concentration in red cell water is only about one half that found in plasma Presumably uric acid enters the cell by diffusion and added urate is distributed in serum and erythrocytes in the same proportion as naturally occurring urate [65]

## SPINAL FLUID

Uric acid is very low in spinal fluid [162] ranging in normal subjects from 0 25 to 1 0 mg per 100 ml The low concentration has been attributed to selective impermeability of the blood brain barrier to urates Values are said to be slightly higher in children (0 3 to 1 5 mg per 100 ml) and to be increased in all forms of meningitis The low levels of uric acid in spinal fluid probably explain the absence of tophaceous deposits in the central nervous system even in advanced cases of gout

Succinoadenine ribonucleoside has recently been identified in spinal fluid [183]

## JOINT FLUID

Uric acid exists in joint fluid in concentrations approximately equal to those of plasma both in normal and hyperuricemic subjects In patients with tophaceous gout uric acid crystals may be present in joint fluid [52]

## GASTROINTESTINAL FLUIDS

Uric acid occurs in variable concentration in saliva gastric and pancreatic secretions intestinal juices and bile Much of the early data on uric acid concentrations in these fluids is reviewed by Bishop and Talbott [63] Recently Sorensen [184] reinvestigated this topic and found values sufficient to account for entrance of about 200 mg uric acid into the gut per day in persons with normal serum values of uric acid

## BODY WATER

The theoretic uric acid space or volume of distribution of the miscible pool of uric acid at a concentration equal to that of plasma is about one half that of total body water [185] This suggests that portions of body water are considerably poorer in uric acid than is plasma One such large compartment may be muscle water in which uric acid concentrations are very low [63] Another smaller compartment is spinal fluid

## URINE

The urine of a normal adult on a low purine diet normally contains 270 to 600 mg uric acid per day Values are somewhat lower in women than in men The mean values ranged from 320 to 583 mg per day in seven published reports summarized by Gutman and Yu [13]

[167] However the author has observed levels ranging from 43 to 68 mg per 100 ml [169] on several occasions in patients with acute leukemia treated with cortisone The possibilities of polymeric complex formation or of unusual binding by abnormal proteins released into plasma in these instances deserve attention

The only other purine base known to exist in plasma is hypoxanthine although small amounts of xanthine may also be present In freshly drawn blood the "oxypurine" concentration ranges from 0 1 to 0 4 mg per 100 ml plasma [170 171] The hypoxanthine in plasma is believed to originate from nucleotides of erythrocytes In blood which has stood for 48 hr a hundredfold increase in hypoxanthine and xanthine has been demonstrated [172] Erythrocytes contain enzymatic equipment to degrade adenyl nucleotides to IMP or adenosine and eventually to hypoxanthine, but since erythrocytes and plasma are devoid of xanthine oxidase activity, hypoxanthine is not further metabolized in blood It is removed either by renal excretion or by extraction by various tissues in which it is then reutilized for nucleoside or nucleotide synthesis or oxidized to uric acid

There is no information to suggest that nucleosides or nucleotides are normal constituents of plasma [173–175] In hypotensive shock resulting from crush injuries to muscle a compound appears in plasma which has many characteristics to suggest that it is inosinic acid [176] Adenylic acid has not been found under these or other circumstances It seems probable that purine bases and ribosides may enter plasma under special circumstances For example excretion of inosine guanosine [177] and adenosine [178] has been observed in urine in certain subjects suggesting prior transport to the kidney by blood plasma Similarly, about ten purine bases regularly appear in urine [151, 179 180] certain of which may show alterations of excretion in various disease states such as gout polycythemia vera leukemia or pernicious anemia It seems reasonable to presume that they exist in plasma in minute concentrations even though they have not yet been demonstrated there Also the purine precursor utilized by bone marrow for purine synthesis postulated by *Lajtha and Vane [125] must be conveyed in the plasma*

## ERYTHROCYTES AND LEUKOCYTES

Under normal circumstances nucleotides of blood exist exclusively in erythrocytes and leukocytes although they have been identified in serum in leukemia and hemolytic anemia [174] The concentration of nucleotides in whole blood normally ranges from 2 to 6 mg nitrogen per 100 ml [181 182] The purine compounds of erythrocytes and leukocytes [127 175] have recently been studied intensively and a variety of compounds identified ATP levels average 433 μmoles and ADP 54 μmoles per liter of whole blood

8 Hydroxy 7 methylguanine does not arise by action of xanthine oxidase on 7 methylguanine [187] so that its origin too is obscure although its structure precludes existence as an N9 ribosyl derivative Of methylated

TABLE 21 2 URINARY EXCRETION IN MILLIGRAMS PER DAY OF PURINE BASES IN NORMAL SUBJECTS

| *Purine base* | *Range* | *Mean* |
|---|---|---|
| Hypoxanthine | 5 9–13 2 | 9 7 |
| Xanthine | 5 1– 8 6 | 6 1 |
| Adenine | 1 1– 1 7 | 1 4 |
| Guanine | 0 2– 0 6 | 0 4 |
| 1 Methylhypoxanthine | 0 2– 0 7 | 0 4 |
| 6 Succinoaminopurine | 0 8– 1 5 | 1 2 |
| 1 + 7 Methylguanine | 5 ?– 7 8 | 6 5 |
| $N^2$ Methylguanine | 0 4– 0 6 | 0 5 |
| 7 Methyl 8-hydroxyguanine | 1 1– 2 0 | 1 6 |

SOURCE B Weissmann et al [151] and [180]

purine bases found in human urine only $N^2$ methylguanine and 1 methyl guanine have been found as trace constituents of nucleic acids in both cases in yeast RNA [145]

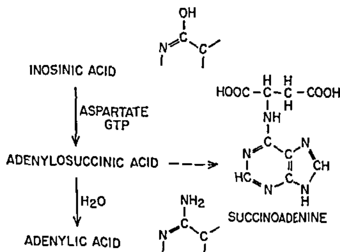



Fig 21 11 Origin of succinoadenine

In addition to the compounds shown in Fig 21 10 at least three unidentified compounds found in human urine appear to be purines on the basis of their absorption spectrums [179] Also in some individuals on a

The urine contains a variety of purine compounds in addition to uric acid although the total of these bases amounts only to some 30 mg per day [*151, 179 180*] The formulas of compounds presently identified and believed to be of endogenous origin, are shown in Fig 21 10 Their normal excretion values are given in Table 21 2

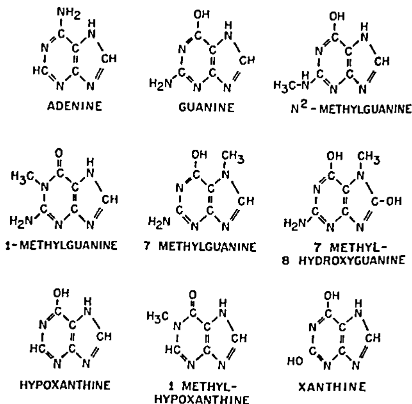



Fig 21 10 Purine bases of human urine Succinoadenine (Fig 21 11) is an additional component

The metabolic origins of some of these compounds are well understood and of others are completely unknown The origins of adenine guanine hypoxanthine and xanthine have already been discussed Succinoadenine is the aglycone of adenylosuccinic acid [*104*] the intermediate in the conversion of IMP to AMP (Fig 21 11) The origins of methylated derivatives are unknown $N^2$ Methylguanine may be formed from guanine at the free base level by a reaction analogous to that forming the N methyl derivative of 2 6-diaminopurine in *Escherichia coli* [*186*] but this is not established It appears likely that methylation of guanine to 7 methyl guanine occurs at the free base level for only in free form does guanine contain a replaceable hydrogen at N7 but again this is not known

addition of unlabeled uric acid may be estimated In the normal subject this addition may be presumed to represent newly synthesized uric acid

In 12 normal male subjects studied by this technique [184 188–193] the miscible pool was an average of 1 176 mg uric acid with values ranging from 866 to 1 587 mg In one normal female the pool was 650 mg [185] These values confirmed earlier analytical values of Gudzent [194]

From the rate of decline in concentration of $N^{15}$ or $C^{14}$ in urinary uric acid it was calculated that from 45 to 85 per cent of uric acid of the miscible pool was normally being replaced each day by newly formed nonisotopic uric acid These values corresponded to quantities of uric acid ranging from 500 to 1 100 mg per day In each case the quantity of uric acid entering the pool exceeded the quantity leaving it in urine by 100 to 260 mg uric acid per day [184 188] The significance of this surplus will be discussed below

Determinations of the miscible pool of uric acid in patients suffering from gout have yielded values ranging from 1 500 to 31 000 mg [184 188 190 192 195] The low value [195] is within the range of the highest values found in normal subjects However in most gouty subjects the miscible pool is decidedly greater than normal the extreme value of 31 000 mg being some twenty five times the average normal value

The miscible pool of uric acid in gouty subjects may be far greater than can exist in solution in body water in view of the known relative insolubility of uric acid in water at physiologic pH and temperature The superficial layers of urate masses situated as tophi may contribute to the miscible pool of uric acid Study of a tophus excised in the course of an isotope experiment in a severely gouty subject demonstrated [190] that peripheral layers of the tophus did indeed contain uric acid enriched with $N^{15}$

The demonstration of large increases in quantities of miscible uric acid present in gouty individuals merely confirms what every clinician already knew and by itself gives no indication of the nature of the disturbance which leads to this increase However the critical information relating to rate of synthesis of uric acid in gouty individuals has proved elusive for in gouty individual dilution of isotope in circulating urate may occur as a consequence of slow exchange with solid urate as well as of addition of newly synthesized urate It is therefore generally not possible to determine the rate of generation of uric acid by the isotope dilution principle in gouty subjects An exception to this statement may exist in those few gouty subjects in whom all of the miscible uric acid may be calculated to be in solution In two such patients Sorensen [184] recently found evidence for increased turnover of uric acid from which he inferred that hyperuricemia was due to increased synthesis of uric acid in these subjects

The *rate of generation* of uric acid has been studied in both normal and

methylpurine free diet the high excretion values of methylated xanthine derivatives suggest that these bases may at times be partly of endogenous origin

## METABOLIC DEFECTS OF GOUT

### ORIGIN OF HYPERURICEMIA

The accumulation of uric acid within the bodies of patients with gout may be considerable even in the absence of clinically detectible tophi or roentgenographic evidence of such deposits In advanced cases uric acid may be present in kilogram quantities Clearly these accumulations of urate must result from a derangement or more than one, in the normal balance between rates of urate formation and rates of urate excretion and destruction The possible causes of elevated urate concentrations in patients with gout have intrigued students of this disease for half a century and evidence has been advanced in support of each theoretically responsible factor Even today no clear cut explanation applicable to all cases can be offered, it may be that so-called primary gout is a heterogeneous disorder In the following sections the available evidence dealing with primary metabolic defects of gout will be considered

*Uric Acid Production*

In the past decade the mechanisms and rates of uric acid production have been under intensive study by means of two general types of biochemical techniques (1) the turnover study employing principles of isotope dilution (2) precursor administration with evaluation of the rate and extent of conversion of the administered substance to uric acid

In the first [*188*] isotopic uric acid is injected intravenously and permitted to mix intimately with uric acid in the body of the subject Urinary uric acid is isolated serially for several days and the isotope concentration of each sample is determined Values are plotted on semi logarithmic coordinates and the theoretical concentration of isotope in the body at the moment of mixing is obtained by extrapolation of the decay curve to zero time The miscible pool of uric acid is defined as that quantity of uric acid in the body of the recipient by which the injected uric acid is promptly diluted The quantity of uric acid present in the miscible pool may be readily calculated from knowledge of the quantity of uric acid injected of the concentration of isotope in it and of the concentration of isotope in the uric acid of the body at the moment of mixing

After mixing has occurred a further progressive decline in concentration of isotope in uric acid occurs because of continuous dilution of the labeled uric acid pool by newly synthesized nonisotopic uric acid molecules From the rate of this decline in isotope concentration the rate of

[197-199] have conformed with those presented above However Bishop Rand and Talbott [198] described one gouty patient who incorporated excessive quantities of glycine $N^{15}$ into urinary uric acid on two of three occasions despite a normal urinary uric acid excretion

In studies [27 200] by the author employing glycine 1 $C^{14}$ 6 of 8 gouty subjects and 1 asymptomatic hyperuricemic patient showed cumulative incorporation of $C^{14}$ into urinary uric acid two to fivefold greater

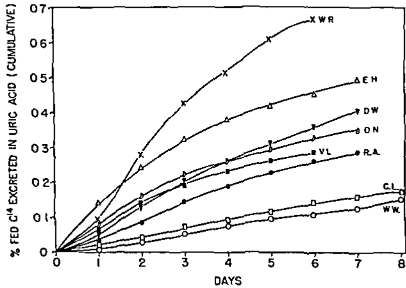



Fig 21 13 Cumulative excretion of C in urinary uric acid following oral administration of glycine 1 C (*From J Clin Invest* 36 1508 1957 *with permission of the publishers*)

than found in 3 control subjects (Fig 21 13) Only 2 of these gouty subjects excreted uric acid in quantities clearly in excess of normal Seegmiller et al [28] and Gutman and associates [199] found normal incorporation values of glycine 1 $C^{14}$ or glycine 2 $C^{14}$ in gouty or asymptomatic hyperuricemic subjects who did not excrete abnormally large quantities of uric acid in urine The results of all published studies employing glycine 1 $C^{14}$ are summarized in Table 21 3A Values for incorporation into uric acid in 7 controls ranged from 0 11 to 0 22 per cent in 7 days (average 0 17 per cent) Values were 0 25 per cent or above in 7 days in 6 of 10 gouty patients and in 1 asymptomatic hyperuricemic subject who excreted *normal* quantities of uric acid in urine under basal conditions In contrast they were 0 25 per cent or more in 7 days in the 3 gouty subjects and the hyperuricemic subject with renal lithiasis who habitually excreted *larger than normal* amounts of uric acid in urine under basal

gouty subjects by observing the rate at which isotope appeared in uric acid when a labeled precursor was administered Studies of this type have now been performed with glycine $N^{15}$, glycine 1 $C^{14}$, glycine 2 $C^{14}$ formate $C^{14}$, 4 aminoimidazole 5 carboxamide 4 $C^{14}$ and 4 $C^{14}$ hypoxanthine 8 $C^{14}$ and adenine 8 $C^{14}$

The first studies were performed by Stetten and associates [195 196] employing glycine $N^{15}$ When isotopic glycine was fed to normal subjects the $N^{15}$ concentration in urinary uric acid rose slowly to a maximum after

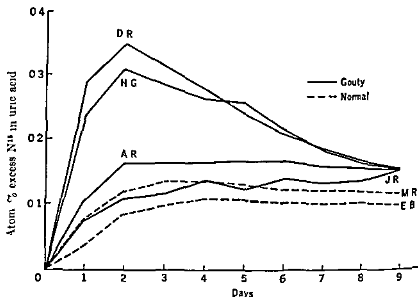



Fig 21 12 Concentration of $N^{15}$ in urinary uric acid following ingestion of glycine $N^{15}$ by normal and gouty subjects (*From J D Benedict et al [196] with permission of authors and publisher* )

3 to 4 days and declined gradually thereafter After 10 days about 0 15 per cent of the administered isotope had been excreted as uric acid Two gouty subjects who consistently excreted excessive quantities of uric acid in urine and therefore could be presumed to be overproducers showed rapid rises in $N^{15}$ concentration in uric acid to values about three times normal and fairly rapid declines in $N^{15}$ concentration during the succeeding days (Fig 21 12) This reflected an exaggeration of the rate of incorporation of dietary glycine nitrogen into uric acid and this finding was borne out by a cumulative incorporation of $N^{15}$ into uric acid three times greater than normal in these subjects However three other gouty subjects whose basal uric acid excretions were within the normal range were also normal as to both rate and magnitude of uric acid labeling

Since these initial studies by Stetten and associates a number of additional studies has been performed with glycine-$N^{15}$ Most of the results

pares gouty subjects as a group with nongouty subjects there is significant overincorporation of glycine 1 $C^{14}$ into urinary uric acid in those with normal as well as in those with excessive urinary excretion of uric acid (Table 21 3B)

It is probable that even those gouty subjects whose incorporation data are not distinguished from normal are in many instances actually overincorporating glycine into uric acid Perhaps when appropriate corrections are made for varying degrees of dilution within the uric acid

TABLE 21 3B STATISTICAL ANALYSIS OF DATA OF TABLE 21 3A

| *Subjects* | *No* | *Incorporation of glycine 1 $C^{14}$ into urinary uric acid per cent in 6–7 days* | *t (vs controls)* | *p (vs controls)* |
|---|---|---|---|---|
| Controls | 8 | 0 17 ± 0 04† | | |
| Gouty normal urate excretion | 11 | 0 29 ± 0 12 | 2 79 | <0 02 |
| Gouty elevated urate excretion | 4 | 0 65 ± 0 36 | 3 87 | <0 01 |

The cumulative incorporation values at 6–7 days have been averaged for three groups controls (including subject L H M whose uric acid values are normal) gouty subjects with normal urinary urate excretions (including subject R A ) and gouty subjects with elevated urinary urate excretion (including subject T J B )

† Mean ± 1 s d

pool and for uricolysis a more definite statement can be made For example a gouty subject (e g L O Table 21 3A) whose apparent glycine incorporation into uric acid was 0 15 per cent in 7 days might in reality have incorporated 0 40 per cent if (1) his miscible pool were twice normal and (2) his uricoly is (i e intestinal de truction of urate) were one-third greater th in normal These are not unreasonable expectations for a gouty patient with a serum uric acid level of 10 6 mg per 100 ml as reported for L O [199]

A second possible explanation for normal incorporation values in some gouty subjects is that the incorporation of glycine may be an inconstant process One patient has already been cited in whom successive glycine-$N^{15}$ studies gave variable results [198] In addition subject O N (Table 21 3 1) was studied on two occasions and showed different results each time An initial study showed an abnormal value of 0 30 per cent in 5 days but when the patient was restudied about one year later he was found to incorporate only 0 17 per cent of a tracer dose of glycine 1 $C^{14}$

A word of explanation is also in order regarding the extraordinary incorporation values found in certain patients of whom W R a mildly gouty subject with a serum uric acid value of 6 4 mg per 100 ml and a urinary excretion of only 313 ± 61 mg per day [200] is perhaps the best example The e re ults under core a disparity encountered occasionally

TABLE 21-3 1 INCORPORATION OF ORAL TRACER DOSE OF GLYCINE 1 $C^{14}$ INTO URINARY URIC ACID IN PATIENTS WITH PRIMARY GOUT

| Clinical status | Subject | Uric acid | | Cumulative recovery of $C^{14}$ in urinary uric acid in percentage of administered $C^{14}$ | | Reference |
|---|---|---|---|---|---|---|
| | | Serum mg/100 ml | Urine (basal) mg/24 hr | 4–5 days | 7 days | |
| Control | C L | 4 4 | 355 | 0 11 | 0 18 | [200] |
| | W W | 4 4 | 346 | 0 09 | 0 15 | [200] |
| | H B | 4 2 | 325 | | 0 22 | [28] |
| | D I | 4 6 | 455 | | 0 21 | [28] |
| | B K | 4 7 | | | 0 16 | [199] |
| | M M | 4 9 | | | 0 13 | [199] |
| | T R | 5 4 | | | 0 11 | [199] |
| Gout (normal uric acid excretion) | O N | 8 5 | 435 | 0 30 | 0 39 | [200] |
| | D W | 8 3 | 317 | 0 31 | 0 40 | [200] |
| | W R | 6 4 | 313 | 0 61 | | [200] |
| | V L | 8 4 | 458 | 0 23 | 0 [illegible]8 (6 days) | [200] |
| | T B | 7 4 | 455 | 0 11 | | [2 ] |
| | R R | 7 2 | 460 | 0 14 | | [2 ] |
| | R W | 7 1 | 463 | | 0 33 | [ 8] |
| | C M C | 5 8–8 0 | 455 | | 0 25 | [28] |
| | O N | 6 6 | 334 | 0 17 | | [28] |
| | R McJ | 9 0 | 513 | | 0 20 | [28] |
| | E H | 7 9 | 514 | | 0 36 | [ 8] |
| | I S | 10 0 | 383 | | 0 14 | [199] |
| | L O | 10 6 | 368 | | 0 15 | [199] |
| | L W | 7 4 | 253 | | 0 06 | [199] |
| Gout (high uric acid excretion) | E He | 8 8→4 5 | 1 054 | | 0 51 | [200] |
| | D R | 10 3 | 689 | | 0 73 | [199] |
| | G M I | 8 7 | 587 | | 0 25 | [28] |
| | H H | 8 0 | 685 | 0 19 | | [200] |
| Asymptomatic hyperuricemia | R A | 7 8 | 343 | | 0 [illegible] | [ 00] |
| Urate renal lithiasis | L H M | 6 1 | 430 | | 0 [illegible] | [28] |
| | T J B | 8 1 | 1 157 | | 1 11 | [28] |

Excretion data on patients from Reference 28 published through the courtesy of Dr J E Seegmiller (personal communication)

conditions It may be concluded that overincorporation of glycine 1 $C^{14}$ into urinary uric acid is a constant finding in hyperuricemic subjects who excrete excessive quantities of uric acid in urine and a frequent though not invariable finding in hyperuricemic subjects who excrete normal quantities of uric acid These data would seem to imply that over production of uric acid from glycine and other small molecular weight precursors is present in a large percentage of gouty subjects including many of those with a normal urinary uric acid excretion When one com

divergent results in these three patients thus appear to parallel the variable results obtained with labeled glycine

**Studies with Aminoimidazolecarboxamide** Seegmiller Laster and Stetten have conducted extensive studies with the purine precursor 4 amino-5-imidazolecarboxamide labeled in C4 with either $C^{13}$ or $C^{14}$ In normal man approximately 20 per cent of administered AIC-$C^{14}$ is excreted in urine as uric acid in 14 days [202] The data show a biphasic

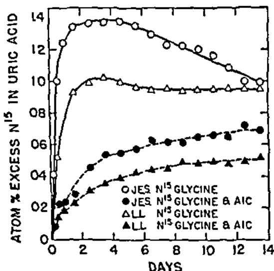



Fig 21 14 Concentration of $N^{15}$ in urinary uric acid in two normal subjects receiving glycine N with and without AIC Note that the concomitant administration of AIC greatly reduced the utilization of glycine N for uric acid synthesis (*From J E Seegmiller et al* [ 0 ] *with permission of authors and publisher*)

incorporation There is a prompt and extensive conversion of ingested AIC $C^{14}$ into uric acid followed by a slower less direct conversion process In all of 5 gouty subjects studied [206 207] incorporation of AIC into uric acid was somewhat greater than normal irrespective of urinary urate excretion The degree of abnormality was magnified when appropriate corrections were made for dilution factors within the urate pool and for uricolysis on the basis of simultaneous studies with uric acid $C^{14}$

In addition these investigators studied simultaneously the incorporation of glycine-$N^{15}$ into urinary uric acid [202] Administration of AIC (in dosage of 0 1 gm per kg) significantly suppressed incorporation of $N^{15}$ into uric acid in both normal (Fig 21 14) and gouty subjects but frequently less effectively in gouty persons These results have important implications for the fundamental nature of the gouty defect as will be seen in a later section

between incorporation values and urinary excretion The factors of concurrent tophus deposition and extrarenal disposal are partial but not adequate explanations for these results A more plausible one is that early incorporation of glycine into uric acid and total production of uric acid do not always bear a *pari passu* relationship one to another A possible explanation of this dissociation based on a variable contribution of the shunt or direct pathway of urate synthesis (see below) to total synthesis has previously been offered [*200*]

Nevertheless despite interpretative difficulties presented by individual studies the data shown in Table 21 34 do provide evidence that glycine incorporation and uric acid production are correlated A plot of the $C^{14}$ incorporation values (6 to 7 days) against the urinary excretion of uric acid including all subjects (control gout urolithiasis) shows a positive correlation having a high degree of statistical significance ($r = 0.7476$ $t = 4.906$ $p = \ll 0.01$) This result provides considerable warrant for regarding the glycine 1 $C^{14}$ incorporation data as generally reflecting closely the production of uric acid and for interpreting overincorporation of this tracer into urinary uric acid as indicative of overproduction

Studies have also been performed with glycine 2 $C^{14}$ in a small number of subjects [*199*] but these suffer from a limitation that also applies to glycine-$N^{15}$ viz, the occurrence of secondary labeling of several atoms of the purine ring other than the one for which the labeled atom is a specific precursor In the case of glycine 2 $C^{14}$ labeling of carbon 2 -4 -6 and -8 occurs in addition to that of carbon 5 which is specifically donated by the $\alpha$ carbon of glycine [*72 201*] In the case of glycine-$N^{15}$ isotope is found in nitrogen atoms other than N7 in appreciable concentration and increasingly so with time [*70 202*] In contrast glycine 1 $C^{14}$ labels C4 of urinary uric acid specifically [*71 72, 200*] Glycine 1 $C^{14}$ has another advantage over glycine-$N^{15}$ as a precursor for human studies in that it can be given in a tracer dose The dose of glycine-$N^{15}$ required 50 to 100 mg per kg is of the order of magnitude of the miscible glycine pool itself [*199 203*] and such loading doses result in two- to tenfold reductions in the percentage of isotope entering uric acid [*199 200*] For these reasons studies with tracer doses of glycine 1 $C^{14}$ have yielded the most reliable information regarding uric acid generation in hyperuricemic subjects

A limited number of studies of rate of generation of urate has also been made with formate $C^{14}$ as the test substance In two gouty subjects who were excreting 830 and 950 mg uric acid daily Spilman [*204 205*] found that both the rate and magnitude of labeling of urinary uric acid were excessive in comparison with two control subjects Subsequently Villa Robecchi and Ballabio [*164*] found no evidence of increased incorporation of formate $C^{14}$ into urinary uric acid in one patient with chronic tophaceous gout and a urinary urate excretion of only 420 mg per day when results were compared with those in one control subject The

uggested that IMP was not the only nucleotide subject to cleavage shortly after formation

In three gouty subjects patterns were obtained in urinary purines following administration of glycine 1 $C^{14}$ which were similar to those in control subjects but there were two potentially important differences (Fig 21 16) Initial hypoxanthine labeling values were never higher than

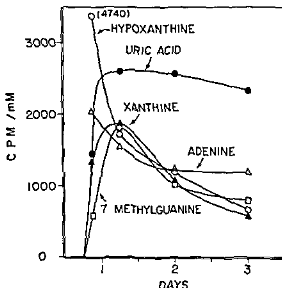



Fig 21 15 Specific activity values of urinary purines in a normal subj ct following oral admini tration of glyc ne 1 C (*From J Cl n I vest* 37 549 1958 *with per miss on of the pu li h* )

those of corresponding uric acid samples and a striking initial labeling of 7 methylguanine was found These results suggested that in gouty subjects the shunt pathway involved a more rapid turnover of the hypoxanthine pool th in in normal subjects and that guanine nucleotides may have been of greater importance as intermediates than in normal persons Since the initial labeling pattern in a patient with myeloid metaplasia resembled that of patients with primary gout especially with reference to labeling of hypoxanthine and 7 methylguanine the pattern in gouty patients was also considered a con equence of overproduction of purine nucleotides and not necessarily indicative of a fundamental difference from normal in pathways available for rapid uric acid synthesis

A study [214] with labeled hypoxanthine demon trated rapid and extensive conversion to uric acid A study with labeled adenine indicated that free adenine was not a significant participant of the shunt pathway in that it was but slowly and paringly converted to uric acid probably

**The Shunt Pathway(s) of Uric Acid Synthesis** In the initial studies of the generation of uric acid from glycine $N^{15}$ the rapidity and extent of overincorporation of isotope into urinary uric acid in gouty hyperexcretors led Stetten [208] to postulate a shunt pathway by which dietary glycine nitrogen could enter the purine nucleus more promptly than in normal man by by passing nucleic acid purines whose turnover appeared to be too slow to account for these results [209] The same reasoning may be employed however to postulate that all subjects possess such a rapid pathway for uric acid synthesis since significant labeling of uric acid occurs within hours in normal and in mildly gouty subjects as well as in severely gouty subjects This is true regardless of the precursor employed although best illustrated following administration of AIC The prompt and excessive incorporation of precursors into uric acid in the gouty subject might be explained either by an accentuation of the normal shunt pathway or by the operation of a new perhaps abnormal pathway also by passing nucleic acids

Stetten [210] drew an interesting analogy between the gouty subject *and the bird which is known to excrete waste nitrogen as uric acid* It does so by a pathway involving rapid synthesis of IMP followed by cleavage reactions yielding hypoxanthine [211] which is readily oxidized to uric acid Stetten suggested this pathway as one possibility to explain rapid and excessive incorporation of glycine-$N^{15}$ into urinary uric acid in certain gouty subjects It would suffice equally well to explain the prompt labeling observed in normal subjects Goodwin [212] has devised a technique for evaluating the magnitude of this shunt pathway based on simultaneous administration of glycine 1 $C^{14}$ and formate-$C^{14}$ and on comparisons of rates of turnover of precursor pools with rates of appearance of isotope in appropriate atoms of urinary uric acid In one normal subject the shunt pathway accounted for synthesis of 70 mg uric acid per day Studies on gouty subjects should prove of great interest

An attempt has been made to study possible intermediates of the shunt pathway in normal and gouty subjects indirectly by measurement of labeling of various urinary purine bases following administration of glycine 1 $C^{14}$ AIC 4 $C^{14}$ hypoxanthine 8 $C^{14}$ or adenine 8 $C^{14}$ Since purine bases do not arise *de novo* but rather by cleavage of the corresponding nucleotide inferences were drawn regarding possible nucleotide intermediates of the shunt pathway

In two normal subjects given glycine 1 $C^{14}$ a very prompt and striking labeling of urinary hypoxanthine resulted and specific activity curves of hypoxanthine and uric acid bore a precursor product relationship [213] (Fig 21 15) These results constitute strong evidence for the operation of the IMP cleavage pathway in normal man In addition the early labeling of such urinary purine bases as adenine 7 methylguanine and xanthine (believed to be derived chiefly from guanine in most subjects)

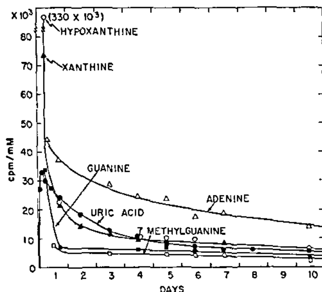



Fig 21 17 Specific a tivity values of urinary purines in a pati nt with cl ronic myelo genous leukemia following intravenous administrati n of AIC 4 C (*From Metabolism* 8 455 1959 *with permission of the publishe s*)

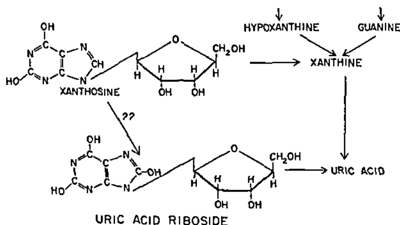



Fig 1 18 U ic aci l ril osi l and a p o il l ute of t ynth

from this ource and one would have to know the ratios of $N^{15}$ and $C^{14}$ in the precur or glycine pool as functions of time in order to interpret the data presented At pre ent therefore an acces ory pathway for uric acid formation is speculative but the re ults pre ented by Laster and Gutman and their coworkers warrant further tudy

via more complex ribosyl intermediates Labeled AIC on the other hand gave rise to prompt and extensive labeling of hypoxanthine xanthine, and uric acid and to a lesser extent also of adenine guanine and 7 methyl guanine (Fig 21-17) These findings strengthened the concept that IMP cleavage was the major but by no means the sole pathway contributing to rapid synthesis of uric acid in normal and gouty man

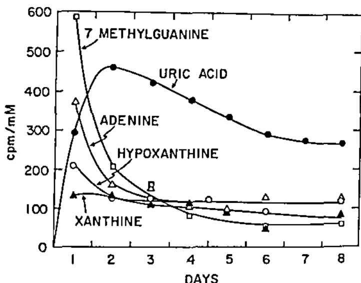



Fig 21-16 Specific activity values of urinary purines in a gouty subject following oral administration of glycine 1 $C^{14}$ (*From J Clin Invest* 37 5[illegible] 1958 *with permission of the publishers*)

The possibility that accessory pathways of uric acid synthesis exist which differ from conventional pathways of nucleotide cleavage and purine base oxidation may also be considered [*215*] Cleavage of uric acid ribonucleoside [*153*] would offer a pathway for synthesis of uric acid not involving xanthine as an immediate precursor Such a pathway would accord nicely with isotopic labeling studies of urinary purines in which hypoxanthine and uric acid were highly labeled but xanthine was not A possible route of synthesis of uric acid ribonucleoside is by direct oxidation of xanthosine (Fig 21 18) but this is not established The role of the ribonucleoside in uric acid synthesis by normal or gouty man remains to be determined

A second possibility regarding accessory pathways of uric acid synthesis has been raised by Gutman and associates [*199*] on the basis of ratios of ($N^{15}$ in N7) to (total $C^{14}$ in uric acid) in excess of 1 in studies in which glycine-$N^{15}$ and glycine 1 $C^{14}$ were administered simultaneously However since glycine $N^{15}$ contributes $N^{15}$ to the general nitrogen pool the newly synthesized glycine reentering the glycine pool may derive $N^{15}$

## TUBULAR REABSORPTION OF URIC ACID

The tubular transport mechanisms for reabsorption of urate though not identified are presumed to be enzymatic Berliner et al [229] have shown that net tubular reabsorption is characterized by a limiting maximal rate of the order of 15 mg per min per 1 73 $m^2$/The magnitude of the *Tm* is such however that it is almost certainly never exceeded under physiologic conditions since at a normal filtration rate of 120 ml per min saturation would not occur below plasma levels of 15 8 to 18 7 mg per 100 ml In patients with depressed filtration rates but normal tubular function even higher values would be required

The normal net clearance of urate is of the order of 7 to 10 ml per min A quantity equal to 5 to 10 per cent of the filtered urate load normally appears in urine [230 231] and this figure is substantially maintained even in the presence of a markedly reduced glomerular filtration rate unless widespread tubular disease is present

In studies of renal clearance of urate a dependency upon rates of urine formation has been observed At urine flow rates below 1 ml per min apparent tubular reabsorption is quantitatively greater than at higher flow rates [231] Beyond 1 to 2 ml per min no further augmentation of clearance occurs [164]

## RENAL EXCRETION OF URIC ACID IN GOUT

A B Garrod first postulated in 1859 that an inherited renal disability in the excretion of uric acid was the cause of the hyperuricemia of gout Numerous later workers accepted and defended this explanation for the hyperuricemia of the disease [232] This conclusion was based on the observation that urinary urate excretion in gouty individuals was generally within the normal range and occasionally even low despite considerable elevation of blood or serum urate levels

With the advent of the clearance concept and the development of techniques for study of discrete renal functions it became possible to evaluate the renal handling of urate by the normal and gouty kidney An impressive number of studies has now been published on this topic Many of these have been reviewed by Gutman and Yu [233] in their notable paper on renal function in gout

Gutman and Yu conducted studies of renal function in some 300 gouty patients and standard renal clearance procedures on approximately 160 cases including some asymptomatic hyperuricemic relatives of these gouty subjects In general glomerular filtration rates ($C_{In}$) renal plasma flow ($C_{PAH}$) and maximal tubular excretory capacity ($Tm_{PAH}$) corresponded with those of normal subjects of equivalent age However impairment of renal hemodynamics (particularly $C_{PAH}$) was not infrequent especially in elderly patients and in those with overt renal dis-

# RENAL HANDLING OF URATE

## TUBULAR SECRETION OF URIC ACID

There is now good evidence for tubular secretion of urate in man and although it has been demonstrated only under quite unphysiologic conditions its very demonstration requires that existing notions regarding urate excretion in normal and gouty man be carefully reevaluated Although tubular secretion of urate was suggested by early workers under the influence of the Cushny school the mechanism of renal handling of urate came to be regarded as exclusively one of glomerular filtration followed by partial tubular reabsorption Because plasma urate is essentially unbound and freely ultrafiltrable complete glomerular filtration has been assumed Existing clearance data have been interpreted as indicating prompt tubular reabsorption of all but 5 to 10 per cent of the filtered urate load The data are equally consistent with quantitative tubular reabsorption of filtered urate followed by tubular secretion of a quantity of urate equivalent to 5 to 10 per cent of the filtered load Finally, they could represent the net result of incomplete tubular reabsorption and tubular secretion The present dilemma renders hazardous too positive a treatment of available data

The occurrence of tubular secretion of urate was demonstrated by Gutman Yu and Berger [216] in subjects who were given a potent uricosuric drug and were rendered markedly hyperuricemic by infusions of lithium urate while subjected to pronounced osmotic diuresis with mannitol In several subjects $C_{u}$ to $C_{i}$ values and excreted urate to filtered urate ratios considerably in excess of 1.0 were achieved These studies impart a new plausibility to an earlier report [217] of $C_{u}$ to $C_{i}$ values ranging from 1.28 to 1.46 in a young man with defective tubular reabsorption of uric acid and indicate that among mammals man as well as the rabbit [218] and the Dalmatian coach hound [219] possess a mechanism for tubular secretion of urate

The demonstration of tubular secretion of urate in man was a logical goal since many observations are difficult to explain on the filtration reabsorption theory alone These include the striking dissociation of filtered urate values and urinary urate excretions [220] the paradoxical effects of uricosuric agents which cause retention of urate when given in small doses [221 222] the profound reduction in renal clearance of urate caused by such substances as pyrazinoic acid [223] pyrazinamide [224 225] chlorothiazide [226] and lactate [227] and the hyperuricemia of toxemia of pregnancy which is greater than can be attributed to glomerular functional changes alone [228] In all these situations variation or inhibition of tubular secretion of urates provides a less awkward interpretation than that of enhancement of tubular reabsorption

gouty patients with normal daily uric acid excretions or those with elevated daily uric acid excretions showed statistically significant difference from control subjects in the percentage of filtered uric acid excreted in urine This difference could be viewed as indicating a more effective tubular reabsorption or less active tubular secretion of uric acid or both

TABLE 21-4A NET TUBULAR REABSORPTION OF URIC ACID IN PRIMARY GOUT

| Controls | | Subjects with gout | | Difference per cent | Significance | Reference |
|---|---|---|---|---|---|---|
| No | Per cent | No | Per cent | | | |
| 61 | 91.9 ± [illegible].3 | 150 | 93.1 ± 2.0 | 1.[illegible] | $p < 0.005$ | [33, 33] |
| 13 | 9[illegible].1 | 9 | 9[illegible].6 | 3.5 | $p < 0.001$ | [240] |
| | 91.7 | 56 | 9[illegible].8 | 4.1 | $p < 0.01$ | [164] |

Values given are percentages of the filtered uric acid load reabsorbed in the renal tubule

TABLE 21-4B NET EXCRETION OF URIC ACID IN PRIMARY GOUT

| Controls | | Subjects with gout | | | Difference per cent | Significance |
|---|---|---|---|---|---|---|
| No | Per cent | Type | No | Per cent | | |
| 61 | [illegible].6 ± 2.4 | A All subjects | 150 | [illegible].8 ± [illegible].1 | 0.8 | $0.0[illegible] > p > 0.01$ |
| 61 | 7.6 ± 2.4 | B Hyperexcretors with GFR > 100 | 40 | 6.6 ± 2.0 | 1.0 | $0.05 > p > 0.0[illegible]$ |
| 61 | 7.6 ± [illegible].4 | C Normoexcretors with GFR > 100 | 54 | [illegible].9 ± 1.7 | 1.7 | $p < 0.01$ |
| 61 | 7.6 ± 2.4 | D Asymptomatic hyperuricemic subjects | 11 | 5.5 ± 0.[illegible]4 | [illegible].1 | $p < 0.01$ |
| | | B vs C | | | 0 | $0.1 > p > 0.05$ |

Values given are percentages of the filtered [illegible] acid load excreted in the urine
SOURCE Recalculated from A B Gutman et al [33]

Recently Nugent and Tyler [241] have published similar data on control and gouty subjects and have shown in addition that nongouty males respond to an increased plasma uric acid concentration with an increase in clearance of uric acid so that at equivalent serum uric acid levels the disparity in $C_{urat}$ to $C_{in}$ ratios between nongouty and gouty subjects is further enhanced Bishop [238] has suggested that the kidney in gout may be limited in its ability to respond to an elevation of plasma uric acid level with an increase in uric acid clearance such as appears to occur in nongouty subjects Critical studies of this point will be of great interest To date the studies on the two groups are not strictly

case The 24 hr urinary urate excretion was *within* the normal range (mean ± s d = 418 ± 70 mg per 26 hr, range = 278 to 558 mg per 24 hr) in 67 0 per cent of patients whereas in 4 3 per cent it was *below* and in 28 7 per cent it was *above* the normal range In 18 0 per cent the values exceeded the normal mean by more than 3 s d ! The filtered urate load calculated as the product of the plasma urate level and $C_{in}$ was found to be increased in most gouty subjects and this was regarded as further evidence that the metabolic defect in gout was prerenal [220]

To this point the e studies are in agreement with earlier studies of the same problem [*234–237*] Of special note are those of Talbott and Coombs [*237*] and of Friedman and Byers [*236*] on young gouty subjects without evidence of renal insufficiency Both groups have emphasized that these subjects had normal urate clearances and excreted greater *than normal quantities of urate in urine A number of the patients studied* by Gutman and Yu showed similar findings These ob ervations are highly significant for they cannot be explained on the basis of the Garrod theory of hyperuricemia and strongly suggest that hyperuricemia is a consequence of overproduction of uric acid in these patients These data tacitly suggest that the finding of a normal or low value of urinary uric acid may be related to the duration of hyperuricemia To some extent this may be true since impairment of renal hemodynamics increases with age However not all young gouty patients show normal urate clearances The author is presently studying two young gouty subjects ages 14 and 21 without significant renal functional abnormalities, whose serum urate levels range from 10 to 12 mg per 100 ml and whose urinary urate values range from 260 to 290 mg per 24 hr The younger patient has had sore heels for 6 months and had a normal serum urate level 1½ years prior to his first symptom Existing data in both subjects suggest defective renal excretion of uric acid

The interpretation of the significance of values for presumptive tubular reabsorption of urate is now difficult In the study by Gutman and Yu the mean net tubular reabsorption was 91 9 per cent of the filtered urate in normal subjects and 93 1 per cent in the gouty subjects The distributions of the two groups overlapped almost wholly Nevertheless the difference in means is highly significant as Bishop has pointed out [*238*] In agreement with this reappraisal certain European investigators have reported greater net tubular reabsorption of urate in gouty subjects than in controls [*164 239 240*] These data are summarized in Table 21-4*A* In Table 21-4*B* the data of Gutman and Yu have been recalculated and are presented in terms of net excretion of uric acid Only those gouty subjects with glomerular filtration rates of 100 ml per min ($C_{in}$) have been included in order to eliminate in so far as possible gross changes perhaps attributable to renal insufficiency All groups of gouty subjects whether asymptomatic hyperuricemic patients *clinically*

studies with labeled and unlabeled uric acid both indicate that a significant quantity of uric acid is disposed of by routes other than the kidney

In initial studies with uric acid $N^{15}$ small though significant concentrations of $N^{15}$ were found in urinary urea and ammonia [188] Subsequently when a relatively large quantity of uric acid $N^{15}$ was administered intravenously to a normal subject [189] highly significant concentrations of isotope were found in urinary urea and ammonia proving

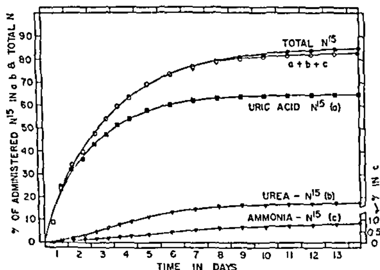



Fig 21 19 Cumulative recoveries of $N^{15}$ in urinary nitrogenous constituents following intravenous administration of uric acid 1 3-$N^{15}$ to a normal subject (From J Biol Chem 203 9 1953 with permission of the publishers)

the occurrence of uricolysis After 2 weeks about 17 per cent of the injected $N^{15}$ was recovered as urea and about 1 per cent as ammonia (Fig 21 19) Following intravenous injection of uric acid $N^{15}$ from 6 to 19 per cent of administered $N^{15}$ was recovered in fecal nitrogen [189 245] In patients with biliary catheters given uric acid $N^{15}$ labeled products were recovered in bile The e results suggested that uricolysis occurred in the intestinal tract but initial attempts to inhibit uricolysis by administration of oral sulfonamide were unsuccessful [189]

Recently Sorensen [184] has repeated these studies using uric acid 2 $C^{14}$ After intravenous administration $C^{14}$ was found in respiratory $CO_2$ urinary urea allantoin and allantoic acid and feces (Table 21 5) The total recovery in degradation products was 22 5 per cent with uric acid $N^{15}$ the comparable value was 25 8 per cent [189] However an interesting difference appeared in the two studies With the passage of

comparable as they have dealt only with nongouty subjects in whom plasma uric acid has been varied relatively acutely and with gouty subjects after years of sustained hyperuricemia

Nugent and Tyler [241] conclude that abnormal renal excretion of uric acid is one important cause of hyperuricemia in some patients with gout It is in all probability at least a major contributing factor but the renal factor fails to offer a satisfactory explanation for the observation that a highly significant minority of gouty patients excrete excessive quantities of urate, an observation that can be explained only by overproduction of uric acid One might intuit that there are two (or more) heterogeneous types of gout one in which hyperuricemia is attributable to abnormal renal excretion of uric acid another in which it is attributable to over production of uric acid However the fact that the group of gouty subjects with excessive urinary urate excretion and the group of gouty subjects with normal uric acid excretion both show a low net urate clearance fails to provide a sound basis for this division These data suggest that abnormal renal excretion of uric acid may be characteristic of primary gout regardless of its stage or of the level of urinary urate but they do not indicate that the renal factor is necessarily the fundamental gouty defect Is it possible that the gouty individual produces in abnormal quantity some substance which has an effect on renal excretion of urate similar to that of lactate? Such an explanation might harmonize the overproduction underexcretion conflict for both might be operating simultaneously

## URICOLYSIS

In a sense gout may be considered a consequence of the genetic deletion of uricase in man for if uricase were present there would be no hyper uricemia and presumably no gout Despite the absence of uricase in human tissues [242 243] uric acid is not the exclusive end product of purine metabolism in man A variety of studies has shown that uric acid is subject to limited catabolism in the human being

Urinary recoveries of injected uric acid are incomplete in normal subjects Thirty five years ago Folin Berglund and Derick [63] and Koehler [244] reported recoveries which ranged from 28 to 91 per cent and averaged about 50 per cent More recently recoveries ranging from 55 to 95 per cent of uric acid $N^{15}$ or 2 $C^{14}$ have been reported [184 185 189 191 193 245] The average of 14 studies with uric acid $N^{15}$ was 75 6 per cent

Studies of turnover of uric acid in normal man have uniformly shown that the quantity of uric acid synthesized per day is greater than the quantity appearing in urine [184 188] In studies with uric acid $N^{15}$ [185] or $C^{14}$ [184] the fraction of the turnover appearing in urine is essentially the same as the fraction of injected uric acid recovered in urine Thus

are shown in Table 21 5 where it may be seen that the quantity of $C^{14}$ recovered in various degradation products was reduced from 22 5 to 3 0 per cent during drug treatment Moreover during intestinal bacteriostasis uric acid was found in feces whereas none was found in the initial experiment An average of 186 mg per day of uric acid or 26 6 per cent of the total turnover was excreted in feces over a 5 day period during bacteriostasis a figure agreeing well with the recovery of 30 1 per cent of administered $C^{14}$ in stools There can no longer be much doubt that intestinal bacterial degradation is the major cause of uricolysis in man A variety of intestinal organisms have the capacity to destroy uric acid particularly *Aerobacter aerogenes* which may constitute 5 per cent or more of the intestinal coliform population [*189*]

Sorensen [*184*] also reinvestigated the content of uric acid in saliva gastric juice and bile and estimated that 100 mg or more enters the alimentary tract each day in these juices An equal quantity may also enter in pancreatic and intestinal juices The quantities of uric acid entering the intestinal tract therefore are larger than previously estimated [*248 249*] and are adequate to account for degradation of one third of the uric acid normally turned over each day

There remains the question of uricolysis within tissues of man Two enzyme systems of human tissues can destroy uric acid in vitro at physiologic pH viz verdoperoxidase [*250*] and cytochrome cytochrome oxidase [*251*] Additionally Bien and Zucker [*252*] have shown that leukocytes and erythrocytes of normal subjects will destroy uric acid during prolonged incubations and Villa Polli and Bussi [*252a*] have demonstrated uricolytic properties of leukocyte extracts This activity resides primarily in cells of the myeloid series [*253*] which are known to contain verdoperoxidase [*254*] Canellakis Tuttle and Cohen [*250*] have shown that the products of peroxidative destruction of uric acid in phosphate buffer are chiefly urea and allantoin In a recent study of the mechanism of peroxidatic uricolysis Howell has found that the apoenzyme is unnecessary and that the prosthetic group hematin actively destroys uric acid in the presence of hydrogen peroxide yielding allantoin and other products [*255*]

In Sorensen s experiments [*184*] the excretion of $C^{14}$ in urea and allantoin was relatively little changed during intestinal bacteriostasis despite profound alterations in the amounts of $C^{14}$ found in other degradation products (Table 21-5) This suggested that allantoin and possibly some of the urea were *not derived from intestinal breakdown of uric acid* *The* common denominator of mammalian systems capable of destroying uric acid is the presence of a hematin prosthetic group Since a small but detectable steady state concentration of hydrogen peroxide exists in living tissue (Chap 46) it may be that a nonenzymatic hematin peroxidatic destruction of uric acid proceeds to a limited extent in human tissue

time following injection of isotopic uric acid an increasing fraction of $N^{15}$ was found in urinary compounds other than uric acid, reaching 50 per cent on the tenth day whereas the amount of $C^{14}$ in urinary urea averaged only 2 5 per cent of $C^{14}$ in uric acid throughout the experiment This difference is probably attributable to late recycling of $N^{15}$ into urinary urea a process minimized with $C^{14}$ in which respiratory $CO_2$ constitutes the chief breakdown product $CO_2$ accounts for 10 to 16 per cent of $C^{14}$ administered as uric acid 2 $C^{14}$ in normal man *[184 246]*

TABLE 21 5 RECOVERY OF INTRAVENOUSLY ADMINISTERED URIC ACID 2 $C^{14}$ IN EXCRETORY PRODUCTS (5–10 DAYS) BEFORE AND AFTER ESTABLISHMENT OF EFFECTIVE BACTERIOSTASIS OF THE INTESTINAL TRACT

| *Excretory product* | *Recovery of $C^{14}$ (per cent of dose)* | |
|---|---|---|
| | *Before bacteriostasis* | *During bacteriostasis* |
| Urinary uric acid | 63 0 (10 days) | 55 7 (5 days) |
| Urinary allantoin | 2 1 | 1 8 |
| Urinary allantoic acid | 0 2 | |
| Urinary urea | 2 2 | 0 7 |
| Expired carbon dioxide | 10 9 | 0 5 |
| Fecal products | 7 1 | 0 0 |
| Total recovery in degradation products | 22 5 | 3 0 |

SOURCE L B Sorensen *[184]*

In experiments with uric acid $N^{15}$ about 2 per cent of $N^{15}$ appeared in urinary allantoin *[247]* Following administration of uric acid 2 $C^{14}$ from 0 4 to 4 5 per cent of $C^{14}$ appeared in urinary allantoin and comparable amounts were recovered in urinary allantoic acid *[184]*

## THE SITES OF URICOLYSIS

When labeled uric acid was administered *orally* to normal subject only 9 to 11 per cent was absorbed and excreted unchanged in urine *[184 191]* With uric acid $N^{15}$ 47 per cent of the $N^{15}$ was recovered in urinary urea in 3 days *[191]* With uric acid 2 $C^{14}$ only 2 4 per cent appeared in urea in 6 days *[184]* Fifty five per cent of the $C^{14}$ was excreted as respiratory $CO_2$ in 2 days An additional 16 3 per cent was recovered in feces 83 to 91 per cent of this amount being found within the intestinal bacteria themselves Only a trivial amount was found in fecal uric acid These findings implicated intestinal bacteria as the agents responsible for the breakdown of the orally administered uric acid

To verify the role of the intestinal flora in uricolysis in man the degradation of *intravenously* administered uric acid was studied in a normal subject before and after an effective bacteriostasis was achieved with concomitant sulfonamide streptomycin and neomycin *[184]* The results

Secondary gout occurs as a complication of certain disorders of the hematopoietic system in which, as a consequence of accelerated turnover of nucleic acids there is an overproduction of uric acid and other purines Talbott [256a] and Gutman and associates have recently reviewed their experiences in this disorder [257-259] It occurs with some frequency in myeloid metaplasia [258] in 5 to 9 per cent or more of patients with polycythemia vera (especially in those cases merging into the phase of myeloid metaplasia) [260 261] occasionally in secondary polycythemia [262] chronic myelogenous leukemia acute leukemia pernicious anemia Cooley s anemia other chronic hemolytic anemias in adults [263] and multiple myeloma [264] The greatest incidence is in those disorders grouped by Dameshek [265] as the myeloproliferative syndromes

The common denominator of these disorders is hyperuricemia consequent to increased formation and degradation of nucleic acids The term *secondary gout* is presently reserved for those patients who develop clinical manifestations of gout acute or chronic responsive to the usual therapeutic measures This restriction in the use of the term is not altogether satisfactory for the predisposition toward development of clinical gout may be present even in hyperuricemic individuals of this group who never develop symptoms of gout just as it is presumed to exist in hyperuricemic members of gouty families all of whom are believed to carry the *gouty trait*

In many cases clinical gout in a patient with a myeloproliferative disorder may represent chance concurrence of primary gout with a second relatively common disease There are a number of differences between primary and secondary gout however which suggest that secondary gout occurs as an acquired disorder Its incidence in polycythemia vera 2 to 10 per cent is too high to be due to chance The incidence in females in the study by Yu and Gutman of 20 cases [259] was much higher (30 per cent) than in 540 cases of primary gout (3 per cent) and the mean age of onset of overt gout was much higher (54 vs 40 years) Moreover there may be a distinct temporal relationship to therapy such as radiation or chemotherapy in polycythemia or leukemia or administration of liver or vitamin $B_{12}$ in pernicious anemia And finally familial occurrence is not conspicuous [257]

The serum level of uric acid in patients with secondary gout tends to be higher than in those with primary gout The mean value in the series of Yu and Gutman [259] was 12 0 mg per 100 ml compared with a mean of 9 1 mg per 100 ml in primary gout The mean urinary excretion of uric acid was also greater 634 compared with 497 mg per day Excretion of uric acid varies quite widely among these patients depending in part upon the type of hematopoietic disturbance present [266]

Urinary excretion of purine bases has also been studied in selected patients with disorders of the type associated with secondary gout [267]

The extent of this reaction probably would not exceed *2 to 4 per cent* of the uric acid turnover per day as a maximal value The urinary allantoin values normally some 10 to 70 mg per day reflect in large part ingested allantoin *[247]* On a ' purine free diet (of necessity also allantoin poor) Wiechowski *[256]* found allantoin excretion to be not more than 11 mg per day This value may approximate the endogenous allantoin production and is within the range of the turnover percentage suggested above

### URICOLYSIS IN GOUT

Decreased uricolysis has been proposed as a cause of hyperuricemia in gout *[63 252]* However recoveries of injected unlabeled uric acid have ranged from 15 to 94 per cent in the urine of gouty subjects *[63, 244]* Recoveries of injected uric acid $N^{15}$ *[245]* or $C^{14}$ *[184]* have ranged from 34 to 54 per cent in urine All these values are lower, on the average than those found in nongouty subjects Furthermore in gouty subjects the fraction of the daily turnover of uric acid recovered in urine is smaller than in normal subjects These studies are complicated by the presence of solid urate with which labeled urate may exchange *[190]* but evidence in other studies has also suggested that extra renal disposal of uric acid is greater than normal in gouty subjects *[184]* Indeed uricolysis should be accentuated in hyperuricemic individuals since in hyperuricemia a larger than normal quantity of uric acid would enter the intestinal tract Pollycove et al *[246]* have reported a greater than normal yield of $C^{14}O_2$ from injected uric acid $C^{14}$ in hyperuricemic subjects

Bien and Zucker *[252]* observed less destruction of uric acid by whole blood white or red blood cells of gouty subjects than of normal persons *during a 24 hr incubation study For example a mean decrease of 34 4 ±* 3 2 per cent was observed in plasma urate after 24 hr incubation of normal whole blood compared with 23 2 ± 4 2 per cent in blood from gouty subjects Since only slight uricolysis occurred during the initial 6-hr period the destruction observed may have been due to factors not operating in vivo The maximal uricolysis attributable to the tissues of man 2 to 4 per cent of the uric acid turnover is too small to be compatible with these results Thus no substantial evidence exists to implicate failure of uricolysis in the hyperuricemia of gout On the contrary emphasis *should be placed* upon enhanced *enteral uricolysis as a com*pensatory factor in gout tending to lessen hyperuricemia and perhaps constituting the major process of disposal of uric acid in patients with severe renal insufficiency

## SECONDARY GOUT

A discussion of secondary gout is warranted in this chapter because of the light it may help throw on metabolic problems of primary gout

In the patient with myeloid metaplasia given glycine 1 $C^{14}$ [*213*] the labeling of intermediary purines in urine clearly showed that processes of urate formation could be di sected into two cla ses—those yielding urate by rapid pathways manifested by maximal enrichment of pre cursor purines in a matter of hours and those yielding urate by lower pathways reflected in attainment of secondary maxima in precursor purines some days later (Fig 21 20) The major difference between these

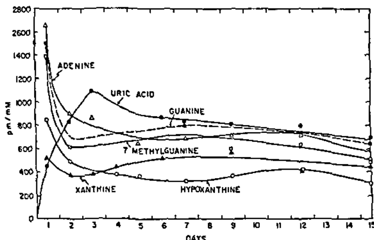



Fig 21 0 Specif c activity values of urinary purines in a patient with myeloid meta plasia following oral administrat on of glycine 1 C (*From Metabolism 6 44 1957 with pe mission of the publishers*)

patterns and ones obtained in normal subjects or patients with primary gout lay in the great quantitative accentuation of the slower pathway as evaluated by inspection of enrichment curves of precursor purines In harmony with these results and interpretations are data presented by Weissmann Bromberg and Gutman [*1.1*] on enrichment of precursor purines in urine following admini tration of glycine-$N^{15}$ to a patient with polycythemia

The e data illustrate clearly that the predominant pathways of uric acid bio ynthesis are different in primary and secondary gout Patients with myeloproliferative disorders are di tinguished from normal subjects and patients with primary gout by an accelerated and augmented turn over of nucleic acids According to pre ent concepts however a large portion of pathways involved in this derangement of purine metabolism is common al o to normal persons and patients with primary gout viz the reactions generating purine nucleotides from small molecular weight precursors and those involved in nucleotide interconversions and break down and oxidation of bases to uric acid

In general, these patients tend to show normal or low values of hypoxanthine, xanthine and 1 + 7 methylguanines and normal or high values of adenine, guanine 7-methyl 8 hydroxyguanine, and succinoadenine In addition increases of urinary inosine and guanosine and of certain other purine and pyrimidine compounds have been noted in leukemic subjects [177]

The miscible pool of uric acid and its turnover have been determined in only a few subjects of this category Both were increased in one leukemic and one of two polycythemic patients studied by Bishop Garner and Talbott [192] In contrast a larger number of studies of purine production from labeled precursors has been carried out The rate curves of glycine-$N^{15}$ incorporation into uric acid in primary and secondary polycythemia [262, 267] and in myeloid metaplasia [268] reveal a slowly progressive enrichment with maxima appearing from 10 to 14 days after glycine ingestion in contrast to the early peak found in normal subjects and patients with primary gout Cumulative incorporation of $N^{15}$ into uric acid was greater in patients with abnormalities of the myeloid series as in primary polycythemia and myeloid metaplasia than in patients with polycythemia secondary to congenital heart disease The presence of secondary gout introduced no distinguishing features in the curves of glycine $N^{15}$ enrichment The late maxima observed were presumably related to abnormally great turnovers of nucleic acids Time relationships appear to be consistent with this interpretation for RNA and some fractions of DNA [209] are known to have turnover times of the order of 2 weeks

In patients of this category given glycine $N^{15}$ the concentration of isotope in urinary uric acid was distinctly less in the first few days than in normal man or in patients with primary gout Yu et al [267] interpreted this as indicating a diversion of glycine from direct to indirect metabolic pathways of incorporation into uric acid However if this were true the *cumulative* incorporation of isotope should also be less than normal during the first few days and this was not found Moreover when glycine 1 $C^{14}$ was given in tracer doses to a patient with polycythemia vera and secondary gout and to another with myeloid metaplasia the initial specific activity values in urinary uric acid were of the same order as those found in control subjects and the cumulative total incorporation of $C^{14}$ into uric acid was greater in both patients from the outset [213] In addition the urinary purine bases were very highly labeled on the first day following glycine 1 $C^{14}$ administration in the patient with myeloid metaplasia Even more striking labeling was observed in urinary purines especially hypoxanthine and xanthine in a matter of hours following administration of AIC 4 $C^{14}$ to a patient with chronic myelogenous leukemia (Fig 21 17) [214] These results illustrate the active operation of several shunt pathways involving various nucleotides in these subjects

individuals predisposed toward development of clinical gout because of inheritance or a blood dyscrasia acute gout is the exception and is sporadic and unpredictable in its occurrence there is no correlation between the uricosuric effect of a drug and its usefulness in acute gout there is no consistent change in the serum or urinary uric acid preceding during or following an acute attack of gout the precipitation of acute attacks by surgery or the rebound attacks following cessation of ACTH or steroid therapy are not easily explained on the basis of effects upon uric acid metabolism per se Additional arguments have been presented by Hench [47] and by Gutman and Yu [13]

Since overproduction of uric acid has been demonstrated in both primary and secondary gout it is logical to turn suspicion upon known precursors of uric acid which are common intermediates in both types of exaggerated uric acid production Presumably these would include the precursors of purine nucleotides their interconversion products and their degradation products but not those solely concerned with nucleotide–nucleic acid pathways Since acute gouty arthritis is characterized by florid inflammatory and pronounced local vasomotor changes it is pertinent to consider the propensities of purine compounds to produce such changes Uric acid itself is relatively inert pharmacologically except for effects caused by its precipitation in renal tubules The pharmacologic effects of purine bases ribosides and ribotides have been thoroughly reviewed by Drury [270] Guanine and hypoxanthine together with their respective ribosides and ribotides have little pharmacologic action Xanthine also is relatively inert except for its mild diuretic effect Adenine in small doses has little effect other than mild leukocytosis although in larger doses it is highly toxic to the kidney because of deposition of a highly insoluble oxidation product 2 8-dihydroxyadenine [271] However adenosine and its various phosphorylated derivatives are known to have potent cardiovascular effects when injected in small doses including peripheral vasodilation and local inflammatory reactions These actions of adenyl compounds are impressive enough to have prompted the suggestion that they may play a role in the production of inflammation following injury [270] Despite the variety of effects produced by these substances nothing suggesting acute arthritis has yet been attributed to adenyl compounds except by Thannhauser and Bommes [272] and efforts by Gutman and Yu [13] to confirm these results have been unsuccessful

With the view of exploring the possibility that one immediate cause of acute gouty arthritis whether in primary or secondary gout might be some purine other than uric acid Gutman and associates have studied the excretion of purine bases in urine in a limited number of patients with primary gout during and between acute gouty attacks They [180 ?58] reported that excretion of succinoadenine is elevated during interval

The addition of a greatly expanded nucleic acid purine pool, or at least of a nucleic acid pool with a greatly accelerated turnover need not therefore introduce any new reactions into the metabolic schemes already discussed The likelihood that the intermediates employed in overproduction of uric acid in both primary and secondary gout are to a large extent common ones despite the difference in etiology and pathogenesis may eventually permit construction of a unified concept regarding derangements responsible for acute gouty attacks in these two forms of gout

## ACUTE GOUT

A century ago A B Garrod proposed that acute gout was caused by crystallization of urate in tissue and that the inflammatory reaction and paroxysm of gout were consequences of this process

There is surprisingly little direct evidence for or against this interpretation although there is considerable inferential evidence against it It is true that urate crystals may be found within the synovium of a joint involved in its first acute gouty attack but some synovial biopsy samples obtained during an attack have failed to disclose crystals Conversely urate deposits may be found in synovia of joints which have never been the site of acute gout and large periarticular accumulations may develop with only indolent reaction on the part of the tissue

Typical attacks of acute gouty arthritis occur most commonly in a genetically predisposed male with preexisting hyperuricemia They also occur in hyperuricemic patients with excessive turnover of nucleoprotein purines i e in secondary gout Recently, acute attacks have also been reported in patients without known predisposition from either of these factors during treatment with pyrazinamide [*269*] or chlorothiazide [*226*] two agents which produce hyperuricemia through effects upon renal excretion of uric acid The common denominator of acute gouty arthritis would therefore seem to be hyperuricemia but this too appears to have certain exceptions Many qualified observers have encountered occasional patients in whom unquestionable acute attacks have occurred in the presence of normal serum uric acid levels as determined by reliable methods Also there is a tendency for patients with gout to experience more frequent acute attacks shortly after beginning the regular use of probenecid, while the serum uric acid level is low

Additional observations tending to cast doubt upon the direct or exclusive role of uric acid per se in the acute gouty attack include the following intravenous infusion of uric acid in doses of 20 to 100 mg per kg in normal [*229*] or gouty [*63*] persons will not precipitate an acute attack nor will oral feeding or even subcutaneous injection of uric acid around joints [*13*] acute gouty arthritis occurs rarely if at all in chronic nephritis with hyperuricemia and nitrogen retention in hyperuricemic

Levin and associates [778 279] made observations during and between 12 attacks in 6 gouty patients and conducted metabolic balances in 2 subjects for 90 and 161 days respectively during spontaneous acute attacks and during attacks induced by withdrawal of ACTH and testosterone The results indicated no evidence of pituitary or adrenocortical hypofunction in relation to acute attacks of gout and no significant differences between the responses of normal and gouty patients to administration or withdrawal of ACTH Neither did these studies support the concept of a ' gout cycle with respect to electrolyte metabolism [237] Nevertheless speculation continues that temporary relative adrenocortical deficiency or diminished target responsiveness may be implicated in the pathogenesis of the acute seizures

### ALLERGY

Many authors have pointed out that certain aspects of the acute gouty attack resemble those of an allergic reaction These are reviewed in detail by Gutman and Yu [18] and by Bauer and Calkins [30] and the inadequacies of the hypersensitivity theory are emphasized Recently Villa Robecchi and Ballabio [164] have again suggested allergy as an explanation of the inflammatory reaction of acute gout while attributing the hyperuricemia and tissue accumulations of uric acid to concomitant increases of production and of tubular reabsorption of urate

## METABOLIC ASPECTS OF THE TREATMENT OF GOUT

The objectives of treatment differ according to the stage of the disease From the standpoint of therapy there are four stages of primary gout asymptomatic hyperuricemia acute gouty arthritis interval gout and chronic tophaceous gout In addition special therapeutic considerations exist in secondary gout and in patients who form uric acid stones whether these are idiopathic or secondary to renal tubular disease or to primary or secondary gout

### ACUTE GOUT

It is convenient to consider first the treatment of the acute gouty attack since most commonly it is this event which first brings the patient to the physician The objective is termination of the attack in the shortest time possible by the mildest means available Several drugs are useful for this purpose viz colchicine phenylbutazone corticotropin and cortisone or related steroid products

*Colchicine*

The dramatic effect of colchicine upon the acute gouty attack is not accompanied by any detectable influence upon uric acid metabolism

phases but declines during acute gout whereas that of 7 methyl-8-hydroxyguanine is normal during interval gout and rises during an attack. They suggested that acute gout is accompanied by an imbalance between adenyl and guanyl nucleotides but cautiously refrained from further speculation as to the significance of these observations Of interest however is the observation that 7 methyl 8-hydroxyguanine is consistently elevated in patients with myeloproliferative disorders predisposing toward development of secondary gout [267] Further observations on the excretion of purine bases in all phases of primary and secondary gout as well as in patients with unrelated types of inflammation will be of great interest

Because of the highly specific effect of colchicine in acute gout knowledge of its locus of action may constitute an important clue to the metabolic defect underlying the acute gouty attack Recently, Laster and Blair [103] have demonstrated that colchicine inhibits uric acid riboside phosphorylase apparently on a competitive basis Phenylbutazone another drug effective in acute gout, also did so but less effectively while probenecid which is without effect upon acute gout did not inhibit the phosphorylase These results must be interpreted with caution at this time since the role and indeed the occurrence, of uric acid riboside is not established in man

ROLE OF THE PITUITARY ADRENAL SYSTEM IN ACUTE GOUT

Since acute gout may be precipitated by such diverse factors as local trauma surgical procedures physical overexertion infection emotional upsets, and dietary and alcoholic overindulgence it was a logical development of the unifying concept of stress as propounded by Selye that a role of the pituitary and adrenal alarm mechanism should be proposed in the initiation of the gouty attack Robinson et al [273] and Hellman [274] have proposed that acute gout is precipitated in the transitory stage of depressed adrenocortical activity presumed to follow cessation of adrenal activation The rebound exacerbation frequently following cessation of ACTH or cortical therapy would appear to be interpretable in similar terms Wolfson et al [275] have proposed a sluggish reactivation mechanism for restoring promptly to normal the secretion of adrenocorticoid following such temporary administration or stimulation of ACTH Additionally Wolfson and Cohn [276] have postulated the secretion of an abnormal *adrenal androgen in gout based on low ketosteroid measurements* in urine in 11 gouty subjects

Gutman and Yu [13] have raised several cogent objections to these theories including the finding of distinctly low urinary excretion of ketosteroids in only 1 of 10 patients with gout A variety of other studies has also indicated that urinary ketosteroids are normal in the majority of patients with primary gout [277]

*Uricosuric Agents*

The advent of clinically useful and well tolerated uricosuric agents has revolutionized the management of gout and the outlook for the gouty patient Chronic tophaceous gout should soon be virtually an unknown disease In the vast majority of gouty patients if the disease is recognized early and managed intelligently disabling tophaceous gout is preventable

The most useful of the uricosuric agents have been probenecid [281–283] and salicylates [284] corticosteroids [192 285–287] and phenylbutazone [288] Of great promise are two recent additions sulfinpyrazone [289] (a phenylbutazone metabolite) and zoxazolamine [290] which are considerably more potent than prior members of this class of agents

The molecular basis for the action of uricosuric agents is unknown and indeed can scarcely be known until the processes involved in uric acid absorption or secretion in the renal tubule are understood Small doses of uricosuric agents cause a reduction of uric acid clearance whereas large doses cause pronounced increases in uric acid clearance This biphasic effect has been attributed to successive inhibition of secretory and reabsorptive processes respectively [220 222a] Striking reductions of the miscible pool of uric acid have been demonstrated after administration of adequate doses of salicylates [190] probenecid [291 292] phenylbutazone [185 288] and cortisone [192] These are probably accounted for entirely by the increment in urate excretion At least in the case of phenylbutazone an increase in uricolysis or of excretion by nonrenal pathways has been excluded No significant increase in synthesis of uric acid was demonstrable when phenylbutazone was given for a few days [185] but in the case of one of two normal subjects given this drug for a longer time and in two hyperuricemic patients given probenecid [291] the increment in uric acid excretion exceeded the measured decrement in the miscible pool of uric acid This suggests either mobilization of uric acid not measured within the pool or a concomitant or subsequent increase in rate of synthesis In the case of corticotropin there is good evidence for increased uric acid production [192 285 287] in addition to the uricosuric effect The possible direct or indirect effects of uricosuric agents on purine synthesis *de noto* and uric acid production deserve further attention

The clinical effectiveness of uricosuric agents in preventing progression of tophi and indeed in mobilizing uric acid deposits is now well known Striking examples of reduction in size of visible tophi and of regeneration and recalcification of bone matrix have been published [281– [illegible]] The shift of uric acid balance in tissue from positive to negative occurs as a consequence of lowering of serum uric acid levels to values sufficiently undersaturated to permit dissolution of tophi In addition the frequency

This drug does not alter serum concentration or renal excretion of uric acid [39] nor does it have any significant effect upon the miscible pool of uric acid or the rate of its turnover [190 193] A clue to its possible mode of action is provided by its inhibition of uric acid riboside phosphorylase [153] but since the function of this enzyme and the existence of its substrate uric acid riboside are not established in man the mechanism of action of colchicine in gout remains an enigma Nevertheless its specificity of action in acute gout provides an important diagnostic test The disadvantage of colchicine is its propensity to cause violent *gastrointestinal upsets when given in doses sufficient to terminate an* acute gouty attack Colchicine itself can be given intravenously with good therapeutic effects and less gastrointestinal toxicity [280] and colchicine by oral or intravenous route is considered the drug of choice for acute gout by many physicians

*Phenylbutazone*

This drug is a potent analgesic agent with remarkable effectiveness in the acute gouty attack [29] It also has potent actions upon renal transport of uric acid When given in adequate dosage phenylbutazone appears to be as effective as colchicine in acute gout and when used for only a few days for occasional attacks is quite safe Its advantage over colchicine is that it causes relief of symptoms without producing violent nausea vomiting or diarrhea Phenylbutazone is also an inhibitor of uric acid riboside phosphorylase

*Corticotropin and Cortical Steroids*

ACTH and cortisone or newer derivatives thereof are effective in acute gout although not so dependably as colchicine or phenylbutazone [29] The mechanism of action is unknown The tendency for a rebound attack to occur upon cessation of therapy has limited enthusiasm for these agents

## CHRONIC TOPHACEOUS GOUT

In chronic tophaceous gout one employs measures to prevent further accumulations of uric acid and to mobilize existing deposits as well as measures to prevent further acute gouty attacks One might add as *objectives the prevention or reversal of accelerated atherosclerosis* or of parenchymal renal disease but at present the influence of therapy upon these processes has not been adequately assessed In interval gout the therapeutic goals and measures employed are similar but frequently less extensive programs are adequate for satisfactory management

The treatment of tophaceous gout consists principally of the use of uricosuric drugs regulation of diet and maintenance colchicine although opinion is divided as to the necessity for and virtues of the last two measures in certain patients

disease and the patient may experience in their place recurring exacerbations of less acute and less paroxysmal joint pain These symptoms too may be lessened by the regular use of colchicine

In a series of 31 gouty patients reported by Gutman and Yu [*29*] daily ingestion of colchicine for 18 months or more resulted in conspicuous reduction *in frequency of attacks in 18 and in 13 instances the response* has made the difference between virtual incapacitation because of frequent interruption of activities and restoration of full employment Talbott s [*294*] experience confirms that presented above

### INTERVAL GOUT

The management of interval gout embodies the principles discussed above except that it is frequently not necessary to institute other than general common sense measures in patients early in the course of the disease For example in a gouty patient just recovered from the initial attack with only a moderately elevated serum uric acid level and no evidence of tophaceous involvement one is justified in withholding further colchicine or in omitting uricosuric agents until more specific indications arise since the interval between the initial and the second attack may be years and no drug is completely nontoxic

### ASYMPTOMATIC HYPERURICEMIA

The incidence of overt gout in hyperuricemic subjects is not known with certainty but may be of the order of 10 to 20 per cent In the remaining group an occasional patient may develop parenchymal renal disease and some will develop renal stones Since the majority of asymptomatic hyperuricemic persons appears to suffer no ill effects from this abnormality no treatment is generally indicated Again high fluid intake control of weight and avoidance of high purine foods would seem only sensible The early recognition of a hyperuricemic male in a family in which severe disabling gout is frequent especially if the gout occurs at an early age would constitute an indication for more extensive prophylactic measure

### SECONDARY GOUT

The treatment of secondary gout is based upon the same principles applied in the management of primary gout with one important potential exception These patients frequently have basal uric acid excretions of considerable magnitude and the administration of a uricosuric agent must therefore be undertaken with caution if at all lest renal stone formation or tubular or ureteral blockade with uric acid occur This is an ever present hazard and also occurs all too frequently following treatment of the primary disease with cancer chemotherapeutic agents radiation [*16* ] or steroids [*169*]

of attacks of acute gout may eventually be considerably reduced in patients receiving uricosuric agents, although shortly after institution of therapy acute attacks may be frequent [283]

Any patient with primary gout who shows clinical or roentgenographic evidence of tophaceous involvement should be treated with an appropriate uricosuric agent In patients with severe renal disease in whom the filtered load of uric acid presented to the tubules is small, use of a potent agent is necessary in order to secure a useful uricosuric effect

*Diet*

Since potent uricosuric agents have become available the necessity for rigid dietary control of purine intake is seldom encountered The major objectives of dietary regulation have therefore become those of common sense such as weight reduction in the large number of gouty patients who are obese and abstinence from high purine foods so as to avoid unnecessary addition of exogenous purine to the uric acid pool In addition, a moderate limitation of protein intake may be advisable since synthesis of uric acid *de novo* is augmented in both normal and gouty subjects by protein loading [129] Patients with moderately restricted consumption of protein and purine show an average decline of serum uric acid level of 1 to 2 mg per 100 ml High fat feedings may influence adversely renal excretion of uric acid [293] through unknown mechanisms Methylxanthines such as caffeine and theophylline are converted in the body to methyluric acids which play no role in gout Therefore coffee tea and other foods or drugs containing methyl xanthines need not be restricted Many patients tolerate alcoholic beverages well Others find beer or wine much more likely to lead to gouty attacks than distilled beverages These are highly individual responses

If the combined use of the general dietary regulations discussed above and a uricosuric agent maintain the serum uric acid level below 5 mg per 100 ml little will be gained by more spartan dietary prescriptions In patients in whom even large doses of uricosuric agents are not adequate to achieve this goal or in whom tophaceous involvement is severe or rapidly progressive a rigid low purine diet may be indicated Diets containing about 30 mg purine N per day are palatable and well tolerated but diets below this figure require omission of most meat and many vegetable items

*Maintenance Colchicine*

The daily administration of small doses of colchicine (0 5 to 2 0 mg) is frequently effective in reducing appreciably the number of acute attacks experienced by the patient with gout In the stage of advanced tophaceous gout acute attacks may be less common than earlier in the course of the

the publication of the historic experiment of A B Garrod in 1848 The first recorded observation of asymptomatic hyperuricemia in a man in whose family gout occurred was made by Folin and Lyman in 1913 [301] In 1931 A E Garrod [302] classified gout as an inborn error of purine metabolism and suggested that gout would probably prove to be due to a dominant Mendelian factor which is expressed fairly commonly as hyperuricemia but only infrequently as gouty arthritis

The occurrence of asymptomatic hyperuricemia among family members of gouty patients was emphasized by Jacobson [11] who examined serums from 1 male relative of each of 3 gouty patients comprising 2 sons and 1

TABLE 21 6 HYPERURICEMIA IN RELATIVES OF GOUTY PATIENTS

| No of gouty patients | No of relatives | No of gouty relatives | No of hyperuricemic relatives | Per cent hyperuricemia | Reference |
|---|---|---|---|---|---|
| 27 | 136 | 3 | 34 | 25 | [303] |
| 55 | 87 | 3 | 21 | 24 | [304] |
| 44 | 136 | 0 | 16 | 11 | [1*] |
| 3 | 29 | 11 | 21 | 72 | |
| 3 | 261 | 16 | 71 | 27 | [15] |

Wilson D Collins D H and Marson R M Gout Discussion Proc Roy Soc Med 44 285 1951

SOURCE C J Smyth [20]

brother and found hyperuricemia in all 3 persons The first extensive study was that of Talbott [303] who reported in 1940 that 25 per cent of 136 asymptomatic blood relatives of 27 gouty patients had hyperuricemia In 20 of 27 families at least 1 person in addition to the index patient had a serum urate value in excess of 6 mg per 100 ml

A number of more recent studies of similar nature has also appeared These are summarized in Table 21-6 The conclusion appears well established that hyperuricemia is a frequent finding among relatives of gouty patients

Smyth Cotterman and Freyberg [304] have plotted their data on serum urate levels in gouty patients and relatives in a frequency diagram All their gouty patients had urate levels of 6 mg per 100 ml or greater The distribution of values among relatives was bimodal for both males and females the nadirs in these distributions being 6 0 and 5 0 mg per 100 ml respectively Using these critical levels they classified 10 of 48 male relatives and 11 of 39 females as hyperuricemic The distribution of hyperuricemic individuals in 19 pedigrees conformed rather closely with expectations if such kindreds were segregated for a dominant autosomal gene for hyperuricemia If this is the mode of inheritance the ratio of hyperuricemic to normal offspring of matings in which only

### RENAL STONES

In patients given uricosuric agents alkali and large fluid intake should also be administered The alkali will increase the solubility of uric acid in urine and may counteract the natural tendency of patients who excrete a highly acid urine [295] to form uric acid stones The bedtime administration of chlorothiazide or acetazolamide may be a useful adjunct in maintaining an alkaline urine

### SUPPRESSION OF URIC ACID SYNTHESIS

Recently azaserine [137] and diazo-oxy norleucine [138] have been shown to reduce the incorporation of labeled glycine into urinary uric acid, presumably by inhibition of purine synthesis *de novo* At present these substances are too toxic and their efforts too difficult to control to provide a useful therapeutic avenue but an attractive goal of the future remains that of pharmacologic regulation of synthesis of uric acid *de novo* in the gouty individual

### DESTRUCTION OF URIC ACID

Recently the infusion of highly purified uricase has been shown to bring about a transient reduction in serum uric acid level in two gouty subjects [296] This demonstration is of theoretic interest but does not constitute a practical form of therapy at present

## GENETIC CONSIDERATIONS

Gout has been recognized as a familial disorder from the dawn of medical history The first recorded expression of this belief is that of *Galen* (A D 131–201) who ascribed gout to debauchery intemperance and an hereditary trait Among ancient and more recent writers Cadogen (1675–1726) stood alone among the many who have written on this topic in believing that gout is not hereditary [9]

Modern studies have recorded a familial incidence of gout ranging from 6 to 81 per cent English observers [297] have reported the familial incidence of overt gout as 38 to 81 per cent in their cases whereas American observers have generally reported lower figures ranging from 6 to 18 per cent in various series [298 299] The reasons for these differences are not entirely clear although the heterogeneity of the American population may make it more difficult to obtain accurate familial data here than in England That these reports need not reflect a true difference in inheritance in the two countries is shown by the data of Talbott of Buffalo who has observed a familial incidence in 75 per cent of his cases [300]

The existence of hyperuricemia in overt gout has been known since

with the enzymatic spectrophotometric method of Kalckar and Praetorius They examined 261 siblings of 32 patients with gout and discovered 71 hyperuricemic individuals as well as 16 definite and 2 doubtful cases of gout among these relatives Plots of serum uric acid values in both controls and relatives of gouty patients described normal distribution curves No clear separation could be made between normal and abnormal uric acid values although the mean values for both male and female relatives of gouty patients were higher than the mean values of their respective control series An attempt was made to fit data on relatives to the sum of two normal distribution curves a fit which should exist if inheritance were of an autosomal dominant pattern The data on male siblings conformed satisfactorily but that on female relatives did not unless it were assumed that the heterozygous state were ten times as common as the homozygous normal state in women an assumption without warrant They therefore concluded that dominance with incomplete penetrance and recessive heredity were excluded and suggested that cumulative gene action (polymerism) might be operative This would mean that the level of uric acid in blood was determined by the cumulative contribution of several perhaps many genes and that hyperuricemic individuals represented the extremes of this trait In this respect hyperuricemia might be compared with tallness a trait for which definition is arbitrary but which is found more frequently in males and is more pronounced in degree in those with a close relationship to tall males The hereditary components in stature are best explained on the basis of an interplay between a large number of genetic factors

The studies of Smyth and Stecher and associates have dealt with different generations within gouty families whereas those of Hauge and Harvald have dealt with siblings of gouty patients Whatever the precise genetic mechanism or mechanisms underlying the gouty defect it is clear that the trait for hyperuricemia behaves as a dominant factor in most gouty families An interesting pedigree of a family probably homozygous for the hyperuricemic trait has been published [13]

It is of interest that the three genetic studies discussed above will permit the interpretation of monomerism and autosomal dominance when the analyses are restricted to males and that Hauge and Harvald have discarded this theory solely on the basis of poor fit of data obtained on females If indeed penetrance is low in women and expressivity hindered it may be very difficult to choose between dominance with failing expressions and polymerism Such situations defy precise genetic characterization at present

An estimate of the relative frequency of hyperuricemia and gout may be made from existing data In 5 series reviewed by Smyth [20] there were 33 definite cases of gout among 163 hyperuricemic relatives representing predominantly American and Danish families One may take

one parent is hyperuricemic should be 1 1 There were no hyperuricemic sons among 14 below the age of 16 years However, when sons above 16 years of age were considered the ratio was 6 hyperuricemic to 7 normal sons Thus the data concerning male relatives were in agreement with the hypothesis of dominant autosomal inheritance if one assumed that the metabolic change resulting in hyperuricemia is one which is not manifested in males until about the age of puberty Among daughters of the same matings 4 were hyperuricemic 10 normal Two of 6 below 16 *years of age and 2 of 4 above 16 years were abnormal* These data on females cannot be used as evidence for an autosomal dominant pattern of inheritance However data in females are less easily interpreted since hyperuricemia frequently does not develop even in genetically endowed individuals until after the menopause

Stecher Hersh and Solomon (*12*) have published data which permit similar analyses A frequency distribution plot of uric acid values of 137 relatives of gouty persons (excluding spouses) was shifted toward high values in comparison with a similar plot of 1 024 determinations on individuals from the general hospital population It was also suggestively bimodal the nadir being 6 0 to 6 4 mg per 100 ml and 23 of 147 determinations on 137 relatives fell above this range

Among relatives they observed hyperuricemia in 15 to 21 per cent of mothers brothers sisters and sons but in none of 45 daughters of index cases These workers tentatively concluded that hyperuricemia was an autosomal dominant trait with low penetrance in both sexes but considerably lower in females than in males When this conclusion was put to numerical test for male offspring only a correction being applied for small family size the expected number of hyperuricemic individuals was 31 Actually 26 were found among 51 men These figures were regarded as showing satisfactory conformity with the expected 1 1 ratio The data were then used to estimate penetrance Since 26 hyperuricemic sons were observed where 31 were expected the penetrance was estimated at about 84 per cent in heterozygote males And since there were only 8 hyperuricemic females compared with 54 hyperuricemic males despite nearly equal sex distribution of the 203 individuals of the study the penetrance was estimated to be about one seventh as high in women as in men or about 14 per cent Since not a single daughter of a gouty patient was found to be hyperuricemic among 45 tested no critical analysis could be applied in the case of female offspring Both these groups agreed that hyperuricemia could not be attributed to a sex linked gene since male male transmission was well documented and frequent Also a recessive genetic transmission pattern was excluded by Stecher et al on the basis of a probability analysis

More recently Hauge and Harvald (*15*) in Denmark have reexamined the problem of inheritance of hyperuricemia All analyses were performed

creased the affinity of PP ribose P amidotransferase for PP ribose-P or decreased its affinity for ATP or ADP Either aberration might permit greater than normal synthesis of phosphoribosylamine despite a normal PP ribose-P to (ATP + ADP) ratio

An increase in the availability of glutamine might push phosphoribosylamine synthesis The effect of high protein diets in augmenting purine synthesis has already been discussed from this viewpoint If there is a greater than normal availability of glutamine for purine synthesis in gout it is not reflected in the plasma levels of glutamine [170]

Overincorporation of glycine 1 $C^{14}$ into urinary uric acid has been demonstrated in a sizable percentage of patients with gout In addition gouty patients show excessive utilization of AIC for uric acid synthesis and administered AIC has less of an inhibitory effect upon glycine incorporation into uric acid in gouty than in normal man

These results have at least one potential common explanation that of a relative excess of the active ribose compound PP ribose-P in the patient with primary gout A high steady state concentration of PP ribose P would result in generation of additional phosphoribosylamine provided that saturation of the amidotransferase had not already been reached by the existing level of PP ribose P This would result in the utilization of a larger than normal quantity of glycine If the various control mechanisms governing interconversion of purine ribonucleotides and synthesis of nucleic acids are competent prompt conversion to uric acid of surplus purine nucleotides would be the anticipated result Under these circumstances the activity of PP ribose-P amidotransferase would still be viewed as controlling the over all rate of purine synthesis *de novo* and the rate would be augmented because of elevation of the PP ribose-P to (ATP + ADP) ratio

A high steady state concentration of PP ribose-P would also permit conversion of a larger than normal quantity of administered AIC to its ribonucleotide by direct reaction of AIC with this active ribose compound Prompt conversion of AIC ribonucleotide to purine ribonucleotides followed by cleavage and oxidation of purine bases to uric acid would result in the appearance of a greater percentage of the test dose of AIC in uric acid in gouty than in normal man In addition under these circumstances a given quantity of AIC would deplete the supply of PP ribose-P less in a gouty than in a normal subject potentially also explaining the smaller reduction in synthesis of uric acid *de novo* from glycine in the gouty subject when AIC is administered

An interesting observation potentially constituting a model for study of mechanisms controlling uric acid synthesis *de novo* is provided by studies of Krakoff and Balis [306] who noted that patients given 2-ethylamino-1 3 4 thiadiazole promptly showed a remarkable increase in serum and urinary levels of uric acid (while also developing toxic lesions within

$^{33}/_{163}$ or one fifth, as an estimate of the proportion of hyperuricemic individuals who at any given time will manifest gout If the estimate [305] is accepted that about 4 per cent of the 12 000 000 arthritic patients in the United States (population, 175 000 000) have gout the incidence of gout would be 274 per 100 000 or 0 27 per cent The frequency of all persons carrying the genetic determinants of hereditary hyperuricemia would then be of the order of 1 4 per cent or 1 in 71 individuals The expected incidence, based upon statistical definition of hyperuricemia would be 2 3 per cent Talbott [39] found 68 hyperuricemic persons among over 1,500 males tested at the time of routine college medical examinations an incidence of about 1 in 22 individuals (4 5 per cent) These values may not be appropriate estimates in countries such as England and India [30 297] where gout is said to be much more common than in the United States and Scandinavia

In the study of Hauge and Harvald [15] no linkage was found between predisposition to hyperuricemia and the genes concerned with blood grouping systems ABO, Rhesus MNS P, Lewis Duffy and Kell

## HYPOTHESIS REGARDING THE FUNDAMENTAL GOUTY DEFECT

In primary gout considerable evidence now points toward overproduction of uric acid as at least one underlying mechanism responsible for hyperuricemia According to present concepts sustained overproduction of uric acid can only occur if there is sustained overproduction of purine nucleotides The key intermediate in the production of purine nucleotides may be phosphoribosylamine for reasons discussed earlier in this chapter

If phosphoribosylamine does occupy such a critical role then the rate of its synthesis may be an important factor in regulating uric acid synthesis. *As a working hypothesis the author would suggest that there is excessive synthesis of phosphoribosylamine in primary gout* The most immediate factors governing the rate of synthesis of phosphoribosylamine will be (1) the activity of its synthesizing enzyme PP ribose-P amidotransferase as determined by the innate characteristics of the enzyme the quantity of enzyme the action of inhibiting nucleotides and perhaps other factors (2) the concentrations of its substrates namely glutamine and PP ribose-P

The synthesis of PP ribose-P amidotransferase is undoubtedly under genetic control The gouty individual might have a greater than normal quantity of the enzyme perhaps if the magnitude of urate synthesis is a *continuous* function having a Gaussian distribution in the general population the gouty subject is one very high on the upper limb of the distribution curve This notion would accord with the genetic data of Hauge and Harvald [13] Or an enzymatic aberration might have in

occur solely as a consequence of renal "retention" of uric acid is difficult to harmonize with existing concepts of the disease.

## SUMMARY

1 Primary gout is a genetically determined disorder of purine metabolism in which the cardinal feature is hyperuricemia. It is preeminently a disorder of adult males, and is characterized both by recurrent attacks of acute arthritis of unknown cause and by a chronic arthritis associated with deposits of urate in joints and other tissues.

2 Hyperuricemia is defined on the basis of statistical analysis of the distribution of serum uric acid values in the general population. In males hyperuricemia is indicated by values above 6 3 mg per 100 ml by colorimetric methods, or above 7 5 mg per 100 ml by enzymatic spectrophotometric methods. In females normal values average about 20 per cent lower than in males.

3 Asymptomatic hyperuricemia is approximately five times as frequent as clinical gout in families of gouty persons. Two types of analysis suggest that the incidence of hyperuricemia is 1 to 2 per cent in the general population. It ranged up to 72 per cent in gouty families.

4 Hyperuricemia cannot be attributed to a defect of uricolysis. Evidence suggests that only 2 to 4 per cent of the turnover of uric acid can be attributed to uricolysis in tissues of man, but that perhaps 25 to 30 per cent can be attributed to bacterial breakdown in the gastrointestinal tract. This fraction is possibly increased in hyperuricemic subjects.

5 The renal mechanism for excretion of uric acid has until recently been viewed as one of glomerular filtration followed by tubular reabsorption of all but 5 to 10 per cent of the filtered urates. Renal tubular secretion has now been demonstrated to occur in man, so that interpretation of clearance data in gouty subjects is uncertain. Values for mean "net" tubular reabsorption of uric acid in gouty subjects are slightly greater than in nongouty subjects at equivalent filtered urate loads. Statistically significant differences in mean values exist between nongouty subjects and asymptomatic hyperuricemic patients, gouty subjects with normal urinary urate excretion, and gouty subjects with habitually excessive urinary urate excretion. In the latter group overproduction of uric acid is virtually certain.

6 Overincorporation of glycine or other precursors into uric acid is demonstrable in many cases of gout. Gouty subjects who excrete abnormally large quantities of uric acid have shown overincorporation of labeled glycine into uric acid whenever studied. These patients are representative of about 28 per cent of the gouty population. Gouty subjects who excrete normal quantities of uric acid have shown overincor

the mouth) The elevation of uric acid production was due to an enhancement of synthesis *de novo* [*306 307*] rather than to acceleration of catabolism of nucleoprotein purine moieties The incorporation curves of labeled formate and glycine into uric acid resembled those seen in gouty subjects who overexcrete uric acid The oral lesions and the increased levels of uric acid were promptly corrected by administration of large doses of nicotinic acid [*306*] or nicotinamide [*307*] and precursor incorporation curves were then again normal Although the mechanisms of these drug actions have not yet been elucidated it is possible that *they involve a common point in the pathways of purine and nicotinic* acid metabolism viz, PP ribose P If thiadiazole blocks endogenous production of nicotinic acid or binding by its ribonucleotide pyrophosphorylase a sparing effect upon PP-ribose P would result and might lead to an augmented synthesis of phosphoribosylamine and purines at the expense of DPN (a possible explanation of the pellagra like mouth lesions) Administration of nicotinic acid might now divert PP ribose-P from purine synthesis toward its normal product nicotinic acid ribonucleotide This explanation though speculative is provocative from the standpoint of the metabolic defect of gout

An increase in the steady state concentration of PP ribose-P at the site of PRA synthesis might derive from one of two major groups of defects (1) those capable of resulting in decreased utilization of a given amount of PP ribose-P in reactions other than that concerned with purine synthesis *de novo* (i e generation of phosphoribosylamine) (2) those capable of resulting in excessive production of PP ribose P In the latter case gout might be viewed fundamentally as a disorder of pentose metabolism rather than intrinsically as a disturbance of purine metabolism

PP ribose-P is generated from ribose 5-phosphate and ATP Ribose 5 phosphate has three primary routes of synthesis overactivity of any of which might result in excessive production of ribose 5 phosphate and of PP ribose P A possible explanation of the common association of hypercholesteremia and hyperuricemia [*308 309*] is that both conditions arise from excessive activity of the phosphogluconic acid oxidation pathway increased generation of reduced TPN leading to enhanced cholesterol synthesis and increased generation of ribose 5 phosphate to enhanced synthesis of PP ribose P phosphoribosylamine and purines

These considerations leave open the possibility that genetic effects at different loci might give rise to a common metabolic disturbance manifested as primary gout caution is urged in adopting too readily a unified genetic concept of the disease In this connection it should be recalled once more that gouty subjects do show a low net renal clearance of uric acid Whether this represents a disturbance related in some way to overproduction of uric acid or whether it is an unrelated derangement cannot now be stated although the concept that primary gout might

3 Wollaston W H On gouty and urinary concretions Phil Tr London 87 386 1 j7
4 Pearson G Experiments and observations tending to show the composition and properties of urinary concretions Phil Tr London 88 15 1 )8
5 Garrod A B Observations on certain pathological conditions of the blood and urine in gout rheumatism and Bright s disease Tr Med Chir Soc Edinburgh 31 83 1848
6 Garrod A B On the blood and effused fluids in gout rheumatism and Bright s disease Tr Med Chir Soc Edinburgh 37 4J 1854
7 Fischer F *Untersuchungen in der Puringruppe* Springer Berlin 1907
8 Folin O and Denis W A new (colorimetric) method for the determination of uric acid in blood J Biol Chem 13 46J 1912-1913
9 Hartung E F Historical considerations Metabolism 6 196 1957
10 Stetten D Jr Gout and metabolism Scient Am 198 73 1958
11 Jacobson B M The uric acid in the serum of gouty and non gouty individuals its determination by Folin s recent method and its significance in the diagnosis of gout Ann Int Med 11 1?7 1937
12 Stecher R M Hersh A H and Solomon W M The heredity of gout and its relationship to familial hyperuricemia Ann Int Med 31 595 1949
13 Gutman A B and Yu T F Gout a derangement of purine metabolism Advances Int Med 5 27 1952
14 Gjorup S Poulsen H and Praetorius E The uric acid concentration in serum determined by enzymatic spectrophotometry Scandinav J Clin Lab Invest 7 201 1955
15 Hauge M and Harvald B Heredity in gout and hyperuricemia Acta med scandinav 152 247 1955
16 Folin O Standardized methods for determination of uric acid in unlaked blood and urine J Biol Chem 101 111 1933
17 Benedict S R The determination of uric acid in blood J Biol Chem 51 187 1922
18 Kalckar H M Differential spectrophotometry of purine compounds by means of specific enzymes I Determination of hydroxypurine compounds J Biol Chem 167 429 1947
19 Praetorius E An enzymatic method for the determination of uric acid by ultra violet spectrophotometry Scandinav J Clin Lab Invest 1 222 1949
20 Smyth C J Hereditary factors in gout A review of recent literature Metabolism 6 218 1957
21 Wolfson W Q Krevsky D Levine R Kodota K and Cohn C Endocrine factors in gout the significance of differences in childhood and adult urate metabolism J Clin Endocrinol 9 666 1949
Wolfson W Q Hunt H D Levine R Guterman H S Cohn C Rosenberg E F Huddleston B and Kadota K The transport and excretion of uric acid in man V A sex difference in urate metabolism with a note on clinical and laboratory findings on gouty women J Clin Endocrinol 9 749 1949
23 Smyth C J Stecher R M and Wolfson W Q Genetic and endocrine determinants of the plasma urate level Science 108 14 1948
24 Talbott J H and Coombs F S Metabolic studies on patients with gout J A M A 110 1 1938
25 Talbott J H Personal communication
26 Goldthwait J C Butler C F and Stillman J S The diagnosis of gout Significance of an elevated serum uric acid value New England J Med 259 1095 1958
27 Wyngaarden J B Normal glycine-C incorporation into uric acid in primary gout Metabolism 7 374 1958
28 Seegmiller J E Laster L and Liddle L W Failure to detect consistent over incorporation of glycine C into uric acid in primary gout Metabolism 7 376 1958
29 Gutman A B and Yu T F Current principles of management in gout Am J Med 13 1? 1952

poration of glycine 1 $C^{14}$ in about one half the cases studied These patients are representative of about 68 per cent of the gouty population Several factors were cited such as greater dilution within an expanded uric acid pool, or greater gastrointestinal loss in the hyperuricemic subjects which might account for an apparently normal glycine incorporation value despite existing overproduction of uric acid Certain studies of 4 amino-5 imidazolecarboxamide incorporation into uric acid and of the rate of turnover of the uric acid pool strongly suggest that overproduction of uric acid occurs in gouty subjects more frequently than can be demonstrated at present by glycine incorporation studies

7 Although studies of renal excretion of uric acid and of glycine incorporation into uric acid in gouty subjects show overlap of results with those of nongouty subjects, the mean values of groups indicate significant abnormalities of both in patients with gout The question is raised whether the metabolic derangement of gout results in excessive production of uric acid and also of some hypothetical substance which interferes with uric acid excretion by the kidney

8 Overincorporation of labeled precursors into uric acid in primary gout appears to occur by way of a shunt pathway, or pathways by which a percentage of newly formed purine nucleotides is broken down to purine bases which are then promptly oxidized to uric acid A hypothesis is presented that overproduction of purine nucleotides occurs because of a defect in regulation of synthesis of phosphoribosylamine the first specific purine precursor Mechanisms may exist for regulation of the rates of synthesis of adenylic and guanylic acids so that excessive synthesis of their mutual precursor, inosinic acid results in cleavage of the surplus and direct synthesis of uric acid

9 The mechanisms underlying the acute gouty attack remain unknown It is not established that crystallization of urate in the synovium is causally related to the paroxysm Demonstration of alterations in urinary excretion of certain purine bases during the acute attack, and of inhibition of uric acid riboside phosphorylase by colchicine, have drawn attention to possible metabolic causes of the attacks Chronic gout is related to accumulations of urate in joints and to associated osteoarthritic changes

10 The heredity of the hyperuricemia of gout is well established Whether the hyperuricemia is determined by an autosomal dominant gene with incomplete penetrance (monomerism) or by a cumulative gene action (polymerism) remains unsettled Indeed if the disorder is heterogeneous, as appears likely different patterns of inheritance may exist

## BIBLIOGRAPHY

1 Sydenham T *Tractatus de podagra et hydrope* G Kettilby London 1683
2 Scheele K W Examen chemicum calculi urinarii Opuscala II 73 1776

3 Wollaston W H On gouty and urinary concretions Phil Tr London 87 386 1797
4 Pearson G Experiments and observations tending to show the composition and properties of urinary concretions Phil Tr London 88 15 1798
5 Garrod A B Observations on certain pathological condition of the blood and urine in gout rheumatism and Bright s di ease Tr Med Chir Soc Edinburgh 31 83 1848
6 Garrod A B On the blood and effused fluids in gout rheumatism and Bright s disease Tr Med Chir Soc Edinburgh 37 49 1854
7 Fischer E *Untersuchungen in der Puringruppe* Springer Berlin 1907
8 Folin O and Denis W A new (colorimetric) method for the determination of uric acid in blood J Biol Chem 13 469 1912-1913
9 Hartung E F Historical con iderations Metabolism 6 196 1957
10 Stetten D Jr Gout and metabolism Scient Am 198 73 1958
11 Jacobson B M The uric acid in the serum of gouty and non gouty individuals its determination by Folin s recent method and its significance in the diagnosis of gout Ann Int Med 11 1277 1937
12 Stecher R M Hersh A H and Solomon W M The heredity of gout and its relationship to familial hyperuricemia Ann Int Med 31 595 1949
13 Gutman A B and Yu T F Gout a derangement of purine metabolism Advances Int Med 5 27 1952
14 Gjorup S Poulsen H and Praetorius E The uric acid concentration in serum determined by enzymatic spectrophotometry Scandinav J Clin Lab Invest 7 201 1955
15 Hauge M and Harvald B Heredity in gout and hyperuricemia Acta med scandinav 152 247 1955
16 Folin O Standardized methods for determination of uric acid in unlaked blood and urine J Biol Chem 101 111 1933
17 Benedict S R The determination of uric acid in blood J Biol Chem 51 187 1922
18 Kalckar H M Differential spectrophotometry of purine compounds by means of specific enzymes I Determination of hydroxypurine compounds J Biol Chem 167 429 1947
19 Praetorius E An enzymatic method for the determination of uric acid by ultra violet spectrophotometry Scandinav J Clin Lab Invest 1 222 1949
20 Smyth C J Hereditary factors in gout A review of recent literature Metabolism 6 218 1957
21 Wolfson W Q Krevsky D Levine R Kodota K and Cohn C Endocrine factors in gout the significance of differences in childhood and adult urate metabolism J Clin Endocrinol 9 666 1949
22 Wolfson W Q Hunt H D Levine R Guterman H S Cohn C Rosenberg E F Huddlestun B and Kadota K The transport and excretion of uric acid in man V A sex difference in urate metabolism with a note on clinical and laboratory findings on gouty women J Clin Endocrinol 9 749 1949
23 Smyth C J Stecher R M and Wolfson W Q Genetic and endocrine determinants of the plasma urate level Science 108 514 1948
24 Talbott J H and Coombs F S Metabolic studies on patients with gout J A M A 110 1977 1938
25 Talbott J H Personal communication
26 Coldthwait J C Butler C F and Stillman J S The diagnosis of gout Significance of an elevated serum uric acid value New England J Med 259 1095 1958
27 Wyngaarden J B Normal glycine-C incorporation into uric acid in primary gout Metabolism 7 374 1958
28 Seegmiller J E Laster L and Liddle L V Failure to detect consistent over incorporation of glycine 1 C into uric acid in primary gout Metabolism 7 376 1958
29 Gutman A B and Yu T F Current principles of management in gout Am J Med 13 744 1952

of synthesis of glycinamide ribotide and its formyl derivative J Biol Chem 221 509 1956
85 Hartman S C Levenberg B and Buchanan J M Biosynthesis of the purines XI Structure enzymatic synthesis and metabolism of glycinamide ribotide and (α N formyl) glycinamide ribotide J Biol Chem 221 105 1956
86 Hartman S C and Buchanan J M Biosynthesis of the purines XXII 2 Amino-N ribosylacetamide 5 phosphate kinosynthase J Biol Chem 233 456 1958
87 Warren L and Buchanan J M Biosynthesis of the purines XIX 2-Amino-N ribosylacetamide-5 phosphate (glycinamide ribotide) transformylase J Biol Chem 229 613 1957
88 Warren L Flaks J G and Buchanan J M Biosynthesis of the purines XX Integration of enzymatic transformylation reactions J Biol Chem 229 627 1957
89 Levenberg B and Buchanan J M Biosynthesis of the purine XIII Structure enzymatic synthesis and metabolism of (α-N formyl)-glycinamidine ribotide J Biol Chem, 224 1019 1957
90 Melnick I and Buchanan J M Biosynthesis of purines XIV Conversion of (α-N formyl)-glycinamide ribotide to (α-N formyl) glycinamidine ribotide purification and requirements of the enzyme system J Biol Chem 225 157 1957
91 Levenberg B and Buchanan J M Biosynthesis of the purines XII Structure enzymatic synthesis and metabolism of 5-aminoimidazole ribotide J Biol Chem 224 1005 1957
92 *Lukens L J and Buchanan J M 5-Amino-4-carboxy imidazoleribotide a new* intermediate in purine biosynthesis Fed Proc 16 214 1957
93 Moat A G Wilkins C N Jr and Friedman H Role for biotin in purine biosynthesis Fed Proc 15 605 1956
94 Lukens L J and Buchanan J M A new intermediate in purine biosynthesis Fed Proc 15 305 1956
95 Flaks J G Warren L and Buchanan J M Biosynthesis of the purines XVII Further studies of the inosinic acid transformylase system J Biol Chem 228 215 1957
96 Flaks J G Erwin M J and Buchanan J M Biosynthesis of the purines XVIII 5-Amino-1 ribosyl-4-imidazolecarboxamide 5 phosphate transformylase and inosinicase J Biol Chem 229 603 1957
97 Stetten M R and Fox C L Jr An amine formed by bacteria during sulfonamide *bacteriostasis J Biol Chem 161 333 1945*
98 Shive W Ackermann W W Gordon M Getzendaner M E and Eakin R E 5(4) Amino-4(5) imidazolecarboxamide a precursor of purines J Am Chem Soc 69 725 1947
99 Miller C S Gurin S and Wilson D W $C^{14}$-Labeled 4(5)-amino-5(4) imidazolecarboxamide in the biosynthesis of purines Science 112 654 1950
100 Schulman M P and Buchanan J M Biosynthesis of the purines II Metabolism of 4-amino-5-imidazolecarboxamide in pigeon liver J Biol Chem 196 513 1952
101 Buchanan J M and Schulman M P Biosynthesis of the purines III Reactions of formate and inosinic acid and an effect of the citrovorum factor J Biol *Chem 202 241 1953*
102 Williams W J and Buchanan J M Biosynthesis of the purine IV The metabolism of 4 amino-5-imidazolecarboxamide in yeast J Biol Chem 202 253 1953
103 Abrams R and Bentley M Transformation of inosinic acid to adenylic and guanylic acids in a soluble enzyme system J Am Chem Soc 77 4179 1955
104 Carter C E and Cohen L H The preparation and properties of adenylosuccinase and adenylosuccinic acid *J Biol Chem 222 17 1956*
105 Joklik W K Adenine succinic acid and adenylsuccinic acid from mammalian liver isolation and identification Biochem J 66 333 1957

106 Lieberman I Enzymatic synthesis of adenosine 5 phosphate from inosine 5 phosphate J Biol Chem 223 327 1956
107 Fromm H J On the equilibrium and mechanism of adenylosuccinic acid synthetase Biochim et biophys acta 29 255 1958
108 Magasanik B Moyed H S and Gehring L B Enzymes essential for the biosynthesis of nucleic acid guanine inosine 5 phosphate dehydrogenase of *Aerobacter aerogenes* J Biol Chem 226 379 1957
109 Lagerkvist U Biosynthesis of guanosine 5 phosphate I Xanthosine 5 phosphate as an intermediate J Biol Chem 233 138 1958
110 Lagerkvist U Biosynthesis of guanosine 5 phosphate II Amination of xanthosine 5 phosphate by purified enzymes from pigeon liver J Biol Chem 233 143 1958
111 Abrams R and Bentley M Biosynthesis of nucleic acid purines III Guanosine 5 phosphate formation from xanthosine 5 phosphate and L-glutamine Arch Biochem & Biophys 79 91 1959
112 Flaks J G Erwin M J and Buchanan J M Biosynthesis of the purines XVI The synthesis of adenosine 5 phosphate and 5 amino-4 imidazolecarboxamide ribotide by a nucleotide pyrophosphorylase J Biol Chem 228 201 1957
113 Lukens L N and Herrington K A Enzymic formation of 6-mercaptopurine ribotide Biochim et biophys acta 24 432 1957
114 Preiss J and Handler P Enzymatic synthesis of nicotinamide mononucleotide J Biol Chem 222 759 1957
115 Kalckar H M The enzymatic synthesis of purine ribosides J Biol Chem 167 477 1947
116 Friedkin M Enzymatic synthesis of desoxyxanthosine by the action of xanthosine phosphorylase in mammalian tissue J Am Chem Soc 74 112 1952
117 Korn E D and Buchanan J M Biosynthesis of the purines VI Purification of liver nucleoside phosphorylase and demonstration of nucleoside synthesis from 4 amino-5-imidazolecarboxamide adenine and 2 6 diaminopurine J Biol Chem 217 183 1955
118 Caputto R The enzymatic synthesis of adenylic acid adenosinekinase J Biol Chem 189 801 1951
119 Kornberg A and Pricer W E Jr Enzymatic phosphorylation of adenosine and 2 6-diamino-purine riboside J Biol Chem 193 481 1951
120 Sandberg A A Lee G R Cartwright G E and Wintrobe M D Purine nucleoside phosphorylase activity of blood I Erythrocytes J Clin Invest 12 1823 1955
121 Goldthwait D Mechanisms of synthesis of purine nucleotides in heart muscle extracts J Clin Invest 36 1572 1957
122 Lowy B W Ramot B and London I M Adenosine triphosphate metabolism in the rabbit erythrocyte in vivo and in vitro Ann New York Acad Sc 75 148 1958
123 Kornberg A Pathways of enzymatic synthesis of nucleotides and polynucleotides in *The Chemical Basis of Heredity* edited by W D McElroy and B Glass p 579 Johns Hopkins Press Baltimore 1957
124 Abrams R and Bentley M Biosynthesis of nucleic acid purines II Role of hypoxanthine and xanthine compounds Arch Biochem & Biophys 58 109 1955
125 Lajtha L G and Vane J R Dependence of bone marrow cells on the liver for purine supply Nature London 182 191 1958
126 Wyngaarden J B Silberman H R and Sadler J H Feedback mechanisms influencing purine ribotide synthesis Ann New York Acad Sc 75 45 1958
127 Bartlett G R Human red cell glycolytic intermediates J Biol Chem 234 449 1959
128 Hurlburt R B Schmitz H Brumm A F and Potter V R Nucleotide metabolism II Chromatographic separation of acid-soluble nucleotides J Biol Chem 209 23 1954
129 Bien E J Yu T F Benedict J D Gutman A B and Stetten D Jr The

relation of dietary nitrogen consumption to the rate of uric acid synthesis in normal and gouty man J Clin Invest 32 778 1953
130 Gots J S Purine metabolism in bacteria V Feedback inhibition J Biol Chem 228 57 1957
131 Way J L and Parks R E Jr Enzymatic synthesis of 5 phosphate nucleotides of purine analogues J Biol Chem 231 467 1958
132 Stetten D Jr Current status of purine biosynthesis—an interim report Ann Rheumat Dis 15 404 1956
133 Magasanik B The metabolic regulation of purine interconversions and of histidine biosynthesis in *The Chemical Basis of Development* edited by W D McElroy and B Glass p 485 Johns Hopkins Press Baltimore 1958.
134 Salser J S and Balis M L Studies on the mechanism of action of 6-mercaptopurine (6MP) in cell free preparations Fed Proc 18 315 1959
135 Silberman H R and Wyngaarden J B Unpublished results
136 Levenberg B Melnick I and Buchanan J M Biosynthesis of the purines. XV The effect of aza L-serine and 6-diazo-5-oxo-L-norleucine on inosinic acid biosynthesis de novo J Biol Chem 225 163 1957
137 Zuckerman R Drell W and Levin M H Urinary purines in gout effect of azaserine Arthritis & Rheumatism 2 46 1959
138 Grayzel A I and Seegmiller J E Suppression of uric acid synthesis in the gouty human by the use of 6-diazo-5-oxo-L-norleucine (DON) J Clin Invest 38 1008 1959
139 Heppel L A and Rabinowitz J C Enzymology of nucleic acids purines, and pyrimidines Ann Rev Biochem 27 613 1958
140 Heppel L A and Hilmoe R J Purification and properties of 5-nucleotidase J Biol Chem 188 665 1951
141 Schmidt G Nucleases and enzymes attacking nucleic acid components in *The Nucleic Acids* edited by E Chargaff and J N Davidson vol I Academic Press Inc New York 1955
142 Nikiforuk G and Colowick S P The purification and properties of 5-adenylic acid deaminase from muscle J Biol Chem 219 119 1956
143 Kalckar H M Differential spectrophotometry of purine compounds by means of specific enzymes III Studies of the enzymes of purine metabolism J Biol Chem 167 461 1947
144 Dunn D B and Smith J D The occurrence of 6-methylaminopurine in deoxyribonucleic acids Biochem J 68 627 1958
145 Adler M, Weissmann B and Gutman A B Occurrence of methylated purine bases in yeast ribonucleic acid J Biol Chem 230 717 1958
146 Littlefield J W and Dunn D B The occurrence and distribution of thymine and three methylated adenine bases in ribonucleic acids from several sources Biochem J 70 642 1958
147 Dunn J and Wyngaarden J B Unpublished results
148 Wilson D Beyer A Bishop C and Talbott J H Urinary uric acid excretion after the ingestion of isotopic yeast nucleic acid in the normal and gouty human J Biol Chem 209 227 1954
149 Roll P M Brown G B DeCarlo F J and Schultz A S The metabolism of yeast nucleic acid in the rat J Biol Chem 180 333 1949
150 Huennekens F M Nurk E and Gabrio B W Erythrocyte metabolism I Purine nucleoside phosphorylase J Biol Chem 221 971 1956
151 Weissmann B Bromberg P A and Gutman A B The purine bases of human urine II Semiquantitative estimation and isotope incorporation J Biol Chem 224 423 1957
152 Bergmann F and Dikstein S Studies on uric acid and related compounds III Observations on the specificity of mammalian xanthine oxidase J Biol Chem 223 765 1956
153 Laster L and Blair A E Phosphorolytic cleavage of uric acid riboside by mammalian enzymes Fed Proc 17 261 1958
154 Friedkin M and Roberts D The enzymatic synthesis of nucleosides. I Thymidine phosphorylase in mammalian liver J Biol Chem 207 245 1954

155 Davis A R Newton F B and Benedict S R The combined uric acid in beef blood J Biol Chem 54 595 192
156 Falconer R and Gulland J M The constitution of purine nucleosides VIII Uric acid riboside J Chem Soc p 1369 1939
157 Carter C F and Potter J L Distribution and properties of uric acid riboside Fed Proc 11 195 1952
158 Overgaard Hansen K and Nielsen A T Does human blood contain uric acid riboside? Scandinav J Clin & Lab Invest 9 194 1957
159 Newton E B and Davis A R Combined uric acid in human horse sheep pig dog and chicken blood J Biol Chem 54 603 1922
160 Bergmann F and Dikstein S The relationship between spectral shifts and structural changes in uric acids and related compounds J Am Chem Soc 77 691 1955
161 Peters J P and Van Slyke D D *Quantitative Clinical Chemistry* 2 d ed vol I p 937 Williams & Wilkins Baltimore 1946
162 Wyngaarden J B Uric acid in *The Cyclopedia of Medicine Surgery Specialties* edited by G M Piersol p 341 Davis Philadelphia 1955
163 Yu T and Gutman A B Ultrafilterability of plasma urate in man Proc Soc Exper Biol & Med 84 21 1953
164 Villa L Robecchi A and Ballabio C B Physiopathology clinical manifestations and treatment of gout Ann Rheumat Dis 17 9 1958
165 Adlersberg D Grishman E and Sobotka H Uric acid partition in gout and in hepatic disease Arch Int Med 70 101 1942
166 Wolfson W Q and Levine R The transport and excretion of uric acid in man IV The renal mechanism for urate excretion Fed Proc 7 136 1948
167 Gudzent F Physikalisch Chemische Untersuchungen uber das Verhalten der Harnsaure in Losungen Ztschr physiol Chem 60 25 1909
168 Talbott J H and Sherman J M Urate distribution in blood J Biol Chem 115 361 1936
169 Gold G L and Fritz R D Hyperuricemia associated with the treatment of acute leukemia Ann Int Med 47 428 1957
170 Segal S and Wyngaarden J B Plasma glutamine and oxypurine content in patients with gout Proc Soc Exper Biol & Med 88 342 1955
171 Jorgensen S and Poulsen H E Enzymic determination of hypoxanthine and xanthine in human plasma and urine Acta pharmacol et toxicol 11 223 1955
172 Jorgensen S Hypoxanthine and xanthine accumulated in stored human blood determination of relative amounts by spectrophotometry Acta pharmacol et toxicol 11 265 1955
173 Kalckar H M Differential spectrophotometry of purine compounds by means of specific enzymes II Determination of adenine compounds J Biol Chem 167 445 1957
174 Nemer H and Stadler F Über den Nucleotidgehalt im Blut von Gesunden und Leukamiekranken Klin Wchschr 27 278 1949
175 Bishop C Rankine D M and Talbott J H The nucleotides in normal human blood J Biol Chem 234 1233 1959
176 Hoffman G T Rottino A and Albaum H G Levels of nucleotide in the blood during shock Science 114 188 1951
177 Adams W S Skoog W A and Davis F W An investigation of purine and pyrimidine excretion in normal and leukemic subjects utilizing ion exchange column and paper chromatographic techniques J Clin Invest 37 875 1958
178 Calvary H O The isolation of adenosine from human urine J Biol Chem 86 63 1930
179 Weissmann B Bromberg P A and Gutman A B The purine bases of human urine I Separation and identification J Biol Chem 224 407 1957
180 Weissmann B and Gutman A B The identification of 6-succino-aminopurine and of 8-hydroxy-7-methylguanine as normal human urinary constituents J Biol Chem 229 239 195

181 Jackson H Jr Studies in nuclein metabolism I Adenine nucleotide in human blood J Biol Chem 57 121 1923
182 Kerr S F and Blish M E A method for the determination of nucleotides in blood and muscle J Biol Chem 98 193 1932
183 Abelskov J Succinyl adenosine a new substance in the human cerebrospinal fluid Biochim et biophys acta 32 566 1959
184 Sorensen L B Degradation of uric acid in man Metabolism 8 687 1959
185 Wyngaarden J B The effect of phenylbutazone on uric acid metabolism in two normal subjects J Clin Invest 34 256 1955
186 Remy C N and Smith M S Metabolism of 2 6-diaminopurine conversion to 5 phosphoribosyl 2 methylamino-6-aminopurine by enzymes of *Escherichia coli* J Biol Chem 228 325 1957
187 Wyngaarden J B 2 6-Diaminopurine as substrate and inhibitor of xanthine oxidase J Biol Chem 224 453 1957
188 Benedict J D Forsham P H and Stetten D Jr The metabolism of uric acid in the normal and gouty human studied with the aid of isotopic uric acid J Biol Chem 181 183 1949
189 Wyngaarden J B and Stetten D Jr Uricolysis in normal man J Biol Chem 203, 9 1953
190 Benedict J D Forsham P H Roche M Soloway S and Stetten D Jr The effect of salicylates and adrenocorticotropic hormone on the miscible pool of uric acid in gout J Clin Invest 29 1104 1950
191 Geren W Bendich A Bodansky O and Brown G B Fate of uric acid in man J Biol Chem 183 21 1950
192 Bishop C Garner W and Talbott J H Pool size turnover rate and rapidity of equilibration of injected isotopic uric acid in normal and pathological subjects J Clin Invest 30 879 1951
193 Buzard J Bishop C and Talbott J H Recovery in humans of intravenously injected isotopic uric acid J Biol Chem 196 179 1952
194 Gudzent F *Gicht und Rheumatismus* Springer Berlin 1928
195 Yu T F Bien L J Gutman A B and Stetten D Jr A further study of the utilization of dietary glycine nitrogen for uric acid synthesis in gout J Clin Invest 32 775 1953
196 Benedict J D Roche M Yu T F Bien E J Gutman A B and Stetten D Jr Incorporation of glycine nitrogen into uric acid in normal and gouty man Metabolism 1 3 1952
197 Muller A F and Bauer W Uric acid production in normal and gouty subjects determined by $N^{15}$ labeled glycine Proc Soc Exper Biol & Med 82 47 1953
198 Bishop C Rand R Talbott J H Rate of conversion of isotopic glycine to uric acid in the normal and gouty humans and how this is affected by vitamin F and folic acid Metabolism 4 174 1955
199 Gutman A B Yu T F Black H Yalow R S and Berson S A Incorporation of glycine-1 $C^{14}$ glycine-2 $C^{14}$ and glycine-$N^{15}$ into uric acid in normal and gouty subjects Am J Med 25 917 1958
200 Wyngaarden J B Overproduction of uric acid as the cause of hyperuricemia in primary gout J Clin Invest 36 1508 1957
201 Llwyn D and Sprinson D B The role of serine and acetate in uric acid formation J Biol Chem 184 465 1950
202 Seegmiller J E Laster L and Stetten D Jr Incorporation of 4-amino-5-imidazolecarboxamide 4-C into uric acid in the normal human J Biol Chem 216 653 1955
203 Crawhall J C and Watts R W E The free glycine metabolic pool in man Biochem J 69 290 1958
204 Spilman E L Uric acid synthesis in the non-gouty and gouty human Fed Proc 13 307 1954
205 Spilman E L Personal communication
206 Seegmiller J E Laster L and Stetten D Jr Uric acid formation in patients with gout The incorporation of 4-amino-5-imidazolecarboxamide-$C^{14}$ into uric

acid Ninth International Congress on Rheumatic Diseases programme vol II p 207 1957
207 Seegmiller J E Personal communication
208 Stetten D Jr Recent contributions to the understanding of the metabolic defect in gout Geriatrics 9 163 1954
209 Furst S S Roll P M and Brown G B On the renewal of purines of the desoxypentose and pentose nucleic acids J Biol Chem 183 251 1950
210 Stetten D Jr On the metabolic defect in gout Bull New York Acad Med 29 51? 1953
211 Greenberg G R De novo synthesis of hypoxanthine via inosine 5-phosphate and inosine J Biol Chem 190 611 1951
212 Goodwin L D Estimation of the rate of synthesis de novo of uric acid in man Arthritis & Rheumatism 2 3?7 195?
213 Wyngaarden J B Blair A E and Hilley L On the mechanism of overproduction of uric acid in patients with primary gout J Clin Invest 37 5 9 1958
214 Wyngaarden J B Seegmiller J E Laster L and Blair A E The utilization of hypoxanthine adenine and 4-amino 5 imidazolecarboxamide for uric acid synthesis in man Metabolism 8 455 1959
215 Wyngaarden J B Intermediary purine metabolism and the metabolic defects of gout Metabolism 6 244 1957
216 Gutman A B Yu T F and Berger L Tubular secretion of urate in man J Clin Invest 38 17?8 1959
217 Praetorius E and Kirk J E Hypouricemia with evidence for tubular elimination of uric acid J Lab & Clin Med 35 865 1950
218 Poulsen H and Praetorius E Tubular excretion of uric acid in rabbits Acta pharmacol et toxicol 10 371 1954
219 Beyer K H Factors basic to the development of useful inhibitors of renal transport mechanism Arch internat pharmacodyn 98 9? 1954
220 Gutman A B and Yu T F Renal regulation of uric acid excretion in normal and gouty man modification by uricosuric agents Bull New York Acad Med 34 283 1958
221 Klemperer F and Bauer W Influence of aspirin on urate excretion J Clin Invest 23 950 1944
222 Yu T F and Gutman A B Paradoxical retention of uric acid by uricosuric drugs in low dosage Proc Soc Exper Biol & Med 90 542 1955
222a Yu T F and Gutman A B Study of the paradoxical effects of salicylate in low *intermediate and high dosage on the renal mechanisms for excretion of* urate in man J Clin Invest 38 1298 1959
223 Yu T F Berger L Stone D J Wolf J and Gutman A B Effects of pyrazinamide and pyrazinoic acid on urate clearance and other discrete renal functions Proc Soc Exper Biol & Med 96 264 1957
224 Cullen J H LeVine M and Fiore J M Studies of hyperuricemia produced by pyrazinamide Am J Med 23 587 1957
225 Shapiro M and Hyde L Hyperuricemia due to pyrazinamide Am J Med 23 596 1957
226 Demartini F E Effect of chlorothiazide on uric acid excretion Program Second Pan American Congress on Rheumatic Diseases Washington D C June 2-6 1959 p 59
227 Yu T F Sirota J H Berger L Halpern M and Gutman A B Effect of sodium lactate infusion on urate clearance in man Proc Soc Exper Biol & Med 96 809 1957
228 Chesley L C and Williams L O Renal glomerular and tubular function in relation to the hyperuricemia of pre-eclampsia and eclampsia Am J Obst & Gynec 50 367 1945
229 Berliner R W Hilton J G Yu T F and Kennedy T J Jr The renal mechanism for urate excretion in man J Clin Invest 29 396 1950
230 Smith H W *The Kidney* Oxford University Press New York 1951
231 Brochner Mortensen K Uric acid in blood and urine Acta med scandinav Suppl 84 1937

232 Thannhauser S J The pathogenesis of gout Metabolism 5 582 1956
233 Gutman A B and Yu T F Renal function in gout with a commentary on the renal regulation of urate excretion and the role of the kidney in the pathogenesis of gout Am J Med 23 600 1957
234 Brochner Mortensen K Diagnosis of gout Acta med scandinav 99 538 1939
235 Coombs F S Pecora L J Thorogood E Consolazio W V and Talbott J H Renal function in patients with gout J Clin Invest 19 525 1940
236 Friedman M and Byers S O Increased renal excretion of urate in young patients with gout Am J Med 9 31 1950
237 Talbott J H and Coombs F S Metabolic studies on patients with gout J A M A 110 1977 1938
238 Bishop C Personal communication
239 Mugler A Pernet A and Friedrich S Le Pouvoir d'épuration du rein pour l'acide urique chez l'hyperuricemique d'après l'etude de 400 clearances in *Contemporary Rheumatology* edited by J Goslings and H Van Swaay p 5 4 Elsevier Amsterdam 1956
240 Sala G Ballabio C B Amira A Ratti G and Cirla E Renal mechanisms for urate excretion in normal and gouty subjects in *Contempo a y Rheumatology* edited by J Goslings and H Van Swaay p 581 Elsevier Amsterdam 1956
241 Nugent C A and Tyler F H The renal excretion of uric acid in patients with gout and in nongouty subjects J Clin Invest 38 1890 1959
242 Jones W *Nucleic Acid. Their Chemical Properties and Physiological Conduct* 2d ed Longmans New York 1920
243 Ro K Über die Urikase J Biochem 14 361 1931–1932
244 Koehler A E Uric acid excretion J Biol Chem 60 621 1924
245 Buzard J Bishop C and Talbott J H The fate of uric acid in the normal and gouty human J Chron Dis 2 4 1955
246 Pollycove M Tolbert B M Lawrence J H and Harman D Uric acid metabolism the oxidation of uric acid in normal subjects and patients with gout polycythemia and leukemia Clin Res Proc 5 38 1957
247 Buzard J A Bishop C and Talbott J H Evidence that urinary allantoin in the human is not derived from body uric acid Fed Proc 13 188 1954
248 Lucke H Das Harnsaureproblem und seine klinische Bedeutung Ergebn inn Med u Kinderh 44 499 1932
249 Kurti L Untersuchungen uber den Harnsaurestoffwechsel bei Nierenkranken Ztschr klin Med 122 585 1932
250 Canellakis E S Tuttle A L and Cohen P P A comparative study of the end products of uric acid oxidation by peroxidases J Biol Chem 213 39 1955
251 Griffiths M Oxidation of uric acid catalyzed by copper and by the cytochrome-cytochrome oxidase system J Biol Chem 197 399 1952
252 Bien E J and Zucker M Uricolysis in normal and gouty individual Ann Rheumat Dis 14 409 1955
252a Villa L Polli E and Bussi L Cited in [164]
253 Ratti G Sull esistenza di una attività uricolitica nell uomo Reumatismo 10 Suppl 2 33 1958
254 Agner K Crystalline myeloperoxidase Acta chem scandinav 12 89 1958
255 Howell R R and Wyngaarden J B Uricolysis by hematin proteins Clin Res 7 297 1959
256 Wiechowski W Das Schicksal intermediarer Harnsaure beim Menschen und der Allantoingehalt des menschlichen Harns nebst Bemerkungen uber Nachweis und Zersetzlichkeit des Allantoins Biochem Ztschr 25 431 1910
256a Talbott J H Gout and blood dyscrasias Medicine 38 1 3 1959
257 Gutman A B Primary and secondary gout Ann Int Med 39 1062 1953
258 Gutman A B Yu T F and Weissmann B The concept of secondary gout relation to purine metabolism in polycythemia and myeloid metaplasia Tr A Am Physicians 69 229 1956
259 Yu T F and Gutman A B Secondary gout—observations in 20 cases

Program Second Pan American Congress on Rheumatic Diseases Washington D C June 2–6 195J p 44
260 Hickling R A Gout leukaemia and polycythaemia Lancet 1 5ɪ 1ɔ53
261 Dimon A and Holub D A Host factors in polycythemia vera Ann Int Med 49 43 1958
26 Yu T F Wasserman L R Benedict J D Bien E J Gutman A B and Stetten D Jr A simultaneous study of glycine N incorporation into uric acid and heme and of Fe utilization in a case of gout associated with polycythemia secondary to congenital heart disease Am J Med 15 845 1953
263 March H W Schlyen S M and Schwartz S E Mediterranean hemopathic syndromes (Cooley s anemia) in adults Am J Med 13 46 19 ?
264 Bronsky D and Bernstein A Acute gout secondary to multiple myeloma a case report Ann Int Med 41 8?0 1954
265 Dameshek W *Leukemia* Grune & Stratton New York 1958
266 Sandberg A A Cartwright G E and Wintrobe M D Studies on leukemia I Uric acid excretion Blood 11 154 1956
267 Yu T F Weissmann B Sharney L Kupfer S and Gutman A B On the biosynthesis of uric acid from glycine N ⁵ in primary and secondary polycythemia Am J Med 21 901 1956
268 Laster L and Muller A F Uric acid production in a case of myeloid metaplasia associated with gouty arthritis studied with N¹ labeled glycine Am J Med 15 857 1ɔ53
269 Gleason D F Street J P and Kahn K A *Effects of pyrazinamide on* serum and urine uric acid J Lab & Clin Med 48 810 195C
270 Drury A N The physiological activity of nucleic acid and its derivatives Physiol Rev 16 ?92 1936
271 Philips F S Thiersch J B and Bendich A Adenine intoxication in relation to in vivo formation and deposition of ? 8-dioxyadenine in renal tubules J Pharmacol & Exper Therap 104 20 195?
272 Thannhauser S J and Bommes A Experimentelle Studien über den Nuclein stoffwechsel II Mitteilung Stoffwechselversuche mit Adenosin und Guanosin Ztschr physiol Chem 91 336 1914
273 Robinson W D Conn J W Block W D and Louis L H Role of the adrenal cortex in urate metabolism and in gout J Lab & Clin Med 33 14 3 1948
74 Hellman L Production of acute gouty arthritis by adrenocorticotrophin Science 109 280 1949
75 Wolfson W Q ACTH and colchicine in the clinical treatment of acute gouty arthritis Physiological considerations of acute gouty arthritis Physiological considerations and review of therapeutic results in fifty-one attacks J Michigan M Soc 49 1058 1950
76 Wolfson W Q and Cohn C The role of the pituitary adrenocorticotropic hormone (ACTH) and of adrenal cortical steroid hormones in the pathological physiology and experimental therapeutics of clinical gout in *Clinical ACTH Conference (First) Proceedings* p ?41 McGraw Hill—Blakiston New York 1950
277 Butt W R and Marson F G W 17 Ketosteroid excretion in gout Brit M J 2 10 3 195?
278 Levin M H Fred L and Bassett S H Metabolic studies in gout J Clin Endocrinol 12 50C 1ɔ5?
279 Levin M H Rivo J B and Bassett S H Metabolic studies in gout with emphasis on the role of electrolytes in acute gouty arthritis Am J Med 15 5 ɔ 1ɔ3
280 Davis J S and Bartfeld H The effect of intravenous colchicine on acute gout Am J Med 16 218 1954
281 Gutman A B and Yu T F Protracted uricosuric therapy in tophaceous gout Lancet 2 1 58 195
282 Bartel E C Treatment of gout Metabolism 6 9ɪ 1957
283 Bartels E C and Matos an G S Gout six year follow up on probenecid (Benemid) therapy Arthritis & Rheumatism 2 1ɔ3 1959

284 Marson F G W Studies in gout with particular reference to the value of sodium salicylate in treatment Quart J Med 22 331 1953
285 Forsham I H Thorn G W Prunty F T G and Hill A G Clinical studies with pituitary adrenocorticotropin J Clin Endocrinol 8 15 1948
286 Ingbar S H Kass E H Burnett C H Relman A S Burrows B A, and Sisson J H The effects of ACTH and cortisone on the renal tubular transport of uric acid phosphorus and electrolytes in patients with normal renal and adrenal function J Lab & Clin Med 38 533 1951
287 Fajans S S Conn J W Johnson D and Christman A A ACTH induced changes in purine and carbohydrate metabolism in Dalmatian and mongrel dogs Endocrinology 49 225 1951
288 Yu T F Sirota J H and Gutman A B Effect of phenylbutazone (3 5-dioxo 1 2-diphenyl-4-N butylpyrazolidine) on renal clearance of urate and other discrete renal functions in gouty subjects J Clin Invest 32 1121 1953
289 Yu T F Burns J J and Gutman A B Results of a clinical trial of G-28315 a sulfoxide analog of phenylbutazone as a uricosuric agent in gout subjects Arthritis & Rheumatism 1 532 1953
290 Burns J J Yu T F Berger L and Gutman A B Zoxazolamine physiological disposition uricosuric properties Am J Med 25 401 1958
291 Bishop C Rand R and Talbott J H The effect of Benemid (p-(di n propylsulfamyl) benzoic acid) on uric acid metabolism in one normal and one gouty subject J Clin Invest 30 889 1951
292 Sirota J H Yu T F and Gutman A B Effect of Benemid (p-(di n propyl sulfamyl) benzoic acid) on urate clearance and other discrete renal functions in gouty subjects J Clin Invest 31 692 1952
293 Lennox W G A study of the retention of uric acid during fasting J Biol. Chem 66 521 1925
294 Talbott J H Gout and gouty arthritis Nursing Outlook 2 540 1954.
295 Henneman P H Wallach S and Dempsey E F The metabolic defect responsible for uric acid renal stone formation J Clin Invest 37 901 1958
296 London M and Hudson P B Uricolytic activity of purified uricase in two human beings Science 125 937 1957
297 Cohen H Gout in *Textbook of the Rheumatic Diseases* edited by W S C Copeman p 361 Livingston Edinburgh and London 1955
298 Neel J V The clinical detection of the genetic carriers of inherited disease Medicine 26 115 1947
299 Rosenberg E F Gout and male hermaphrodism report of a case Ann Rheumat Dis 2 273 1941
300 Talbott J H Gout J Chron Dis 1 338 1955
301 Folin O and Lyman H On the influence of phenylquinolin carbonic acid (Atophan) on uric acid elimination J Pharmacol 4 539 1913
302 Garrod A E Inborn factors in disease Clarendon Press Oxford 1931
303 Talbott J H Serum urate in relatives of gouty patients J Clin Invest 19 645 1940
304 Smyth C J Cotterman C W and Freyberg R H The genetics of gout and hyperuricemia An analysis of nineteen families J Clin Invest 27 749 1948
305 Smyth C J and Huffman E R Gouty arthritis diagnosis and treatment Rocky Mountain M J 52 513 1955
306 Krakoff I H and Balis M E Studies of the uricogenic effect of 2 substituted thiadiazoles in man J Clin Invest 38 907 1959
307 Seegmiller J E Grayzel A I and Liddle L Excessive uric acid production in the human induced by 2-ethylamino-1 3 4-thiadiazole Nature London 183 1463 1959
308 Adlersberg D Newer advances in gout Bull New York Acad Med 25 651 1949
309 Harris-Jones J N Hyperuricemia and essential hypercholesterolemia Lancet 1 857 1957

# Chapter 22

## Xanthinuria*

---

*James B. Wyngaarden*

Xanthinuria is a rare disorder characterized by the excretion of very large amounts of xanthine in urine and a tendency to form xanthine stones. The only well-documented case of xanthinuria is that described in 1954 by Dent and Philpot [1]. Their patient exhibited a striking diminution of uric acid in blood serum and urine. Urinary stones composed of almost pure xanthine were first identified by Marcet in 1817 [2]. Since that time approximately thirty additional cases have been reported [3–13]. Many of these patients had normal blood or serum uric acid levels and therefore were clearly different from the patient of Dent and Philpot.

### CLINICAL FEATURES

*Case of Dent and Philpot [1]*

This patient was a 4½ year old girl with hematuria and urinary frequency. X-ray examination led to a strong suspicion of a nonopaque stone. The urine contained no excess of calcium, cystine, or amino acids. The patient passed a smooth oval stone measuring 1.5 × 1.0 × 0.5 cm and weighing 0.9 gm. It was translucent to x-rays, contained only traces of calcium and magnesium, and was almost ash free. In the murexide test it gave a reddish brown color quite unlike that of uric acid and rather unlike that given by pure xanthine. A hydrolyzed sample yielded traces of amino acids, suggesting that protein was present to the extent of about 5 per cent of the weight of the stone. Scrapings of the stone were dissolved and analyzed by paper chromatography in the presence of various purine markers. The material from the stone matched exactly pure xanthine. The urine was then examined by paper chromatography

* This work was supported in part by grant A 1331 from the National Institutes of Health, Public Health Service.

and shown to contain large quantities of xanthine and no detectable uric acid

Xanthine excretion by the colorimetric method of Williams [14] was about 176 mg per day, or 607 mg per gm creatinine These values suggested that xanthine, not uric acid, was the end product of purine metabolism in this patient (see Figs 21 8 and 22 2) However uric acid may have been present in small amounts for when the urine was analyzed by the method of Folin and Trimble [15] it appeared to contain 30 mg uric acid per day

The plasma was also examined chromatographically It showed neither uric acid nor xanthine even when five times the amount required to show uric acid in normal plasma was employed These results indicated that if either uric acid or xanthine was present the level was less than 1 mg per 100 ml Since normal plasma may contain less than 0 1 mg xanthine per 100 ml, a considerable elevation of this compound might have escaped detection by this method (see below) Standard chemical analysis by the method of Folin and Trimble showed 0 5 mg per 100 ml apparent uric acid

This patient was restudied at age 9 by Dickinson and Smellie [15a] She had had no further calculi in the interim but clubbing of the calices of the left kidney and enlargement of the right kidney had been detected and her blood pressure had varied between 120/65 and 140/80 By specific enzymatic methods plasma oxypurines were 0 74 mg per 100 ml and plasma uric acid was <0 2 mg per 100 ml The renal clearance of oxypurines was 94 ml per min per 1 73 m² body surface area a value equivalent to 82 per cent of the simultaneous endogenous creatinine clearance Fresh urine and plasma contained small quantities of hypoxanthine equivalent to 10 to 20 per cent of xanthine Stored specimens showed larger quantities of hypoxanthine and also showed more uric acid than was found in fresh specimens

*Other Cases of Xanthine Stones*

Xanthine stones have been found in patients ranging from 4 to 72 years of age Three quarters of the 31 reported subjects have been males Two thirds of the xanthine stones have been pure' and one third mixed' The mixed stones have frequently contained uric acid or calcium oxalate or phosphate in addition to xanthine

In no case of xanthine stone other than that of Dent and Philpot has the urinary excretion of xanthine been measured or hypouricemia reported In the few studied cases all adults the level of blood or serum uric acid has been normal [4 10 13] or even somewhat high [9] In one of three Duke Hospital cases hyperuricemia and increased urinary uric acid excretion were found (1958) in a patient who had had a mixed xanthine-uric acid stone 15 years earlier Excretion of xanthine and of

other urinary purines [16] was normal The family also proved to be hyperuricemic A second adult patient who had also passed a mixed xanthine–uric acid stone had a normal uric acid level A third subject a woman who had passed stones since early childhood had pure xanthine stones at age 28 (1947) No uric acid analyses were performed and she has since been lost to follow up (Stone analyses were performed by Heywood Taylor by the murexide method The author is indebted to Dr Taylor for permission to cite these unpublished cases )

An interesting case has been reported by Ichikawa [12] in a 44 year old male who had a left nephrectomy performed after a lengthy history of hematuria and negative preliminary study for urinary calculi The kidney was grossly normal but on palpation was found to be studded with numerous small nodules The cut surface disclosed many small holes with brownish granular concrements which were round or oval smooth and friable Chemical tests revealed these to be pure xanthine and some tubules contained xanthine casts No other instances of parenchymal xanthine deposits have been recorded

An observation of potential significance is that certain of the adults who formed xanthine stones were observed to excrete highly acid urine pH values of 4 0 and 4 9 were recorded in two instances [9 13] In the three Duke Hospital cases urine pH values ranged from 6 0 to 6 5 Xanthine is highly insoluble in acid urine A defect in urinary acidification such as that described by Henneman and coworkers [17] in some uric acid stone formers might have led to xanthine or mixed xanthine–uric acid stone formation in certain of the reported subjects

It is clear that a considerable number of the adults who formed xanthine stones did not have the metabolic defect present in the young girl described by Dent and Philpot The observation that several had normal or even elevated serum uric acid levels probably excludes a block in xanthine oxidation in these subjects Indeed it is not established that they had any defect of xanthine metabolism It may be suspected however that xanthine excretion was elevated in at least one subject whose blood uric acid was high normal for a 60 year old male patient reported by Taylor and Taylor [10] had xanthine stones weighing 12 gm If xanthine excretion were normal in this subject (6 mg per day) these stones would represent quantitative precipitation and retention of the cumulative urinary xanthine excretion of 6 years

Of the 34 known cases of xanthine stones 6 occurred in subjects under the age of 15 In no case was stone formation recorded as a familial tendency and in this group no measurements of serum or urinary xanthine or uric acid were recorded except by Dent and Philpot It is not possible therefore to determine whether any other cases of defect in xanthine metabolism have been observed among xanthine stone formers although the younger patients and those with large pure xanthine stones would

and shown to contain large quantities of xanthine and no detectable uric acid

Xanthine excretion by the colorimetric method of Williams [14] was about 176 mg per day or 607 mg per gm creatinine These values suggested that xanthine not uric acid was the end product of purine metabolism in this patient (see Figs. 21-8 and 22-2) However uric acid may have been present in small amounts for when the urine was analyzed by the method of Folin and Trimble [15] it appeared to contain 30 mg uric acid per day

The plasma was also examined chromatographically It showed neither uric acid nor xanthine even when five times the amount required to show uric acid in normal plasma was employed These results indicated that if either uric acid or xanthine was present the level was less than 1 mg per 100 ml Since normal plasma may contain less than 0 1 mg xanthine per 100 ml a considerable elevation of this compound might have escaped detection by this method (see below) Standard chemical analysis by the method of Folin and Trimble showed 0 5 mg per 100 ml apparent uric acid

This patient was restudied at age 9 by Dickinson and Smellie [15a] She had had no further calculi in the interim but clubbing of the calices of the left kidney and enlargement of the right kidney had been detected and her blood pressure had varied between 120/65 and 140/80 By specific enzymatic methods plasma oxypurines were 0 74 mg per 100 ml and plasma uric acid was <0 2 mg per 100 ml The renal clearance of oxypurines was 94 ml per min per 1 73 m² body surface area a value equivalent to 82 per cent of the simultaneous endogenous creatinine clearance Fresh urine and plasma contained small quantities of hypoxanthine equivalent to 10 to 20 per cent of xanthine Stored specimens showed larger quantities of hypoxanthine and also showed more uric acid than was found in fresh specimens.

*Other Cases of Xanthine Stones*

Xanthine stones have been found in patients ranging from 4 to 72 years of age Three-quarters of the 31 reported subjects have been males Two-thirds of the xanthine stones have been pure and one-third mixed The mixed stones have frequently contained uric acid or calcium oxalate or phosphate in addition to xanthine

In no case of xanthine stone other than that of Dent and Philpot has the urinary excretion of xanthine been measured or hypouricemia reported In the few studied cases all adults the level of blood or serum uric acid has been normal [4 10 13] or even somewhat high [9] In one of three Duke Hospital cases hyperuricemia and increased urinary uric acid excretion were found (1958) in a patient who had had a mixed xanthine–uric acid stone 15 years earlier Excretion of xanthine and of

mixture is carefully evaporated to dryness To the yellow residue are added a few drops of 4 N potassium hydroxide Uric acid forms a purplish violet color at this stage with xanthine the color is a bright red-orange Upon further heating the uric acid sample turns yellow and yields a red-orange residue upon evaporation

The differentiation of xanthine from uric acid is difficult if ammonium hydroxide is used in place of potassium hydroxide as in the conventional murexide test

**Phosphotungstic Acid Test** Xanthine does not give a color test with phosphotungstic acid whereas uric acid yields a deep blue color Xanthine can be carefully oxidized to uric acid with hydrogen peroxide or dichromate [7] and will then give a positive test result

**Folin s Phenol Reagent** Xanthine and guanine react with Folin s phenol reagent to give a blue color but adenine and hypoxanthine do not [23] Uric acid will react but can be destroyed by heating with $HNO_3$ without affecting xanthine

**Spectrophotometric Methods** A small amount of material is dissolved in 0 01 N HCl or 0 01 N NaOH with gentle heat and the spectrum of the solution is examined against an appropriate blank Xanthine exhibits characteristic spectrums as shown in Fig 22 1 [*4] Pure xanthine stones should permit

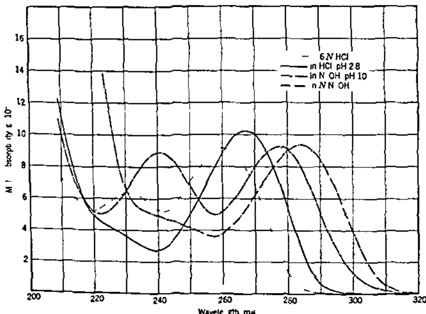



Fig 22 1 Absorpt on spectrums of xa thine (*I pri ted f om Beaver t al* [*] *with permis ion of a th s a d Academic Press Inc*)

provisional identification by spectral examination Upon addition of xanthine oxidase at pH 8 2 the spectrum should change to that of uric acid and by observation at appropriate wavelengths [*5 *6] the quantity of xanthine present may be estimated The presence of xanthine in impure stones particularly those containing uric acid may be difficult to detect

constitute the most interesting group in which to search for such a defect

An early report [18] that serum oxypurine levels are elevated in gout has not been confirmed [19]

*Other Cases of Hypouricemia*

Hypouricemia occurs in association with a number of inborn errors of metabolism but except in the patient described by Dent and Philpot it appears always to be attributable to failure of renal tubular reabsorption of uric acid The classic prototype of such a defect is that of the Dalmatian coach hound in which urate and inulin clearances are identical [20] In man hypouricemia occurs in the de Toni Fanconi syndrome (Chap 37) and in Wilson's disease (Chap 25) as a consequence of the renal lesions of these disorders

An interesting case of hypouricemia has been described by Praetorius and Kirk [21] They discovered a healthy young man with a plasma uric acid value ranging from 0 2 to 0 6 mg per 100 ml and a urinary output of uric acid of 690 mg per day Uric acid clearances ranged from 162 to 284 ml per min (normal = 7 to 10 ml per min) and averaged 46 per cent greater than simultaneous inulin clearances The defect in this subject appears to be that of an extraordinarily high uric acid clearance, possibly including tubular secretion (Chap 21) The original report also describes an elevated level of "oxypurine" in the plasma of this patient, and in that of his father and son both of whom had normal uric acid levels Subsequently the high oxypurine levels in these patients were attributed by other authors [22] to breakdown of red cell nucleotides occurring before plasma was separated from erythrocytes Thus the significance of these elevations of oxypurine levels in this family is unclear

*Characteristics of Xanthine Stones*

The majority of xanthine stones have been described as brownish or brown yellow smooth round or oval friable easily cut with a razor and white and laminated inside A few have been irregular in shape They have ranged in size from a few millimeters in diameter to that of a hen s egg [3] and in weight from a few grains to 3 gm [3] or more [10]

Xanthine stones are radiolucent and are generally discerned on pyelography as filling defects only Occasionally sufficient calcium is entrained within the stone to permit radiographic recognition

A variety of methods has been used for identification of xanthine in the stone Most of these leave much to be desired and it is to be hoped that future identification will be made with the highly sensitive and specific methods now available including differential spectrophotometry and paper chromatography

**Murexide Test** A small amount of the material is placed in a porcelain evaporating dish a few drops of concentrated nitric acid are added and the

tion of uric acid (Fig 22 2) [1] The first oxidation step of hypoxanthine is slower than the second one Nevertheless careful studies in vitro have shown that a small but detectable steady state concentration of xanthine does exist during hypoxanthine oxidation [33] This is greater than can be accounted for by intermediate xanthine bound to the enzyme surface and is sufficiently large to constitute a xanthine pool in solution

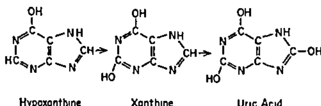



Fig 22 2 Conversion of hypoxanthine to uric acid by xanthine oxidase

Xanthine is formed from guanine by action of guanase Xanthine must then be acted upon by xanthine oxidase to yield uric acid Guanase may be found in tissues which lack xanthine oxidase These include blood [34] and kidney [35] and it is therefore not surprising that urinary xanthine generally appears to have been derived from guanine in isotope labeling studies

Plasma normally contains 0 1 to 0 3 mg per 100 ml hypoxanthine + xanthine [19 27] and investigations by enzymatic differential spectrophotometric methods indicate that hypoxanthine is the major component of this pair [36] Both hypoxanthine and xanthine accumulate in the plasma of shed blood [37] and may be increased 100 fold during standing at room temperature for 48 hr From 10 to 30 per cent of the increment may be due to xanthine which is presumably derived from catabolism of erythrocyte purine compounds [34] Urinary excretion of xanthine normally ranges from 2 to 8 6 (average 6 1) mg per day [30] and is frequently slightly lower in patients with primary gout [38] or with myeloproliferative disorders [39] Xanthine + hypoxanthine excretion is not influenced by probenecid in man [40] This suggests that oxypurine bases are not reabsorbed in the renal tubules by the mechanism responsible for urate reabsorption The clearance of hypoxanthine + xanthine ranges from 15 to 40 ml per min in man and is therefore three to five times the urate clearance

[1] pK for hypoxanthine xanthine and uric acid is 8 8 [illegible] and 5 75 respectively [?] Therefore at pH 7 4 hypoxanthine and xanthine would be present predominantly in their lactam form whereas uric acid would be present chiefly as the monoalkali salt of a mixed lactim lactam form

by these methods although an appreciable increase in absorbance at 292 mμ upon addition of xanthine oxidase would constitute strong evidence for the presence of xanthine since hypoxanthine is so soluble as to be a most unlikely contaminant of the stone One should however ascertain by suitable analyses with uricase [25 27] that the increment in absorbance at 292 mμ does indeed represent generation of uric acid

**Chromatographic Methods** A number of paper [28] and column chromatographic systems [16 29] is available for separation and identification of xanthine Movement of the unknown material in a manner similar to that of known xanthine in several systems would constitute strong evidence for identity of the two substances

A paper chromatographic method was employed by Dent and Philpot in identifying xanthine in the stone recovered from their patient It is hoped that semiquantitative determinations of all the urinary purines by paper [16 30] or column chromatographic methods [16 29] will be performed on subjects who form xanthine stones so that possible lesser alteration of excretions of other purine bases may also be evaluated

## XANTHINE

The origin of xanthine has been discussed in Chap 21 and its relationship to the broader aspects of purine metabolism is presented there In mammalian systems xanthine has three known immediate precursors hypoxanthine guanine and xanthosine (or deoxyxanthosine) and it is the only established precursor of uric acid (Fig 21 8)

Presumably the major precursors of xanthine are hypoxanthine and guanine Each of these bases is formed from its respective nucleoside by action of purine nucleoside phosphorylase an enzyme which readily cleaves inosine and guanosine but has only limited activity toward xanthosine Isotope studies discussed in Chap 21 have provided evidence for significant early labeling of urinary hypoxanthine and guanine following administration of labeled precursors such as glycine or 4 amino-5-imidazolecarboxamide These observations indicate that these purine bases are products of cleavage of newly formed nucleotides as well as *of older nucleotides* derived from catabolism of nucleic acids In most studies the specific activity of urinary xanthine paralleled that of guanine but in some it followed more closely that of hypoxanthine [29 31] Thus the predominant precursor of urinary xanthine may be either guanine or hypoxanthine in different subjects Normally only a very small fraction of the xanthine turnover appears in the urine the largest portion by far serves as a precursor of uric acid

As pointed out above xanthosine and deoxyxanthosine are relatively poor substrates for nucleoside phosphorylase and presumably little xanthine originates from these precursors Xanthine arises from hypoxanthine by action of xanthine oxidase as an intermediate in the forma

TABLE 22 1 RELATIVE RATES OF OXIDATION OF PURINE DERIVATIVES BY BOVINE MILK AND HUMAN LIVER XANTHINE OXIDASES

| *Substrate* | *Product* | *Oxidative pathway*† | *Relative rate with* | |
|---|---|---|---|---|
| | | | *Bovine milk xanthine oxidase* | *Human liver xanthine oxidase* |
| Xanthine | Uric acid | | 1.0 | 1.0 |
| 1 Methylxanthine | 1 Methyluric acid | | 0.45 | 1.0 |
| 6,8-Dioxypurine | Uric acid | | 1.0 | 1.1 |
| Hypoxanthine | Xanthine uric acid (6,8-dioxypurine?) | → 6 → 2,6,8 | 0.7 | 0.7 |
| Purine | Uric acid | → 6 → 2,6 → 2,6,8 | 0.2 | 0.15 |
| 2 Oxypurine | 2,8-Dioxypurine | → 2,8 → 2,6,8 | 0.16 | 0.01 |
| 8-Oxypurine | 2,8-Dioxypurine | → 2,8 → 2,6,8 | 0.015 | 0.007 |
| 8-Oxypurine | Uric acid | | 0.007 | 0.000 |
| 2,8-Dioxypurine | Uric acid | | 0.002 | 0.0005 |

Xanthine served as reference compound

† Indicates order in which the oxidizable compounds were attacked by the enzyme

SOURCE F Bergmann et al [33]

variable rates [52] The enzyme is strongly inhibited by 6-pteridyl aldehyde [53] Xanthopterin is oxidized to its 8 hydroxy derivative by xanthine oxidase [26]

## STUDIES ON THE ACTIVE SITE OF XANTHINE OXIDASE

Xanthine oxidase activity towards purines and aldehydes is inhibited by sulfhydryl poisons and by cyanide A metal mercaptide group has therefore been postulated as an essential part of the active site of xanthine oxidase [54] Cyanide inhibition of xanthine oxidase presumably involves formation of a stable ferric-cyanide or molybdo cyanide complex but *binding studies have clearly shown that such inhibition does not interfere* with the ability of the enzyme to bind purines There is evidence that the isoalloxazine ring of FAD constitutes the purine binding site [33 54] and possibly also the aldehyde binding site [54] The competition by boric acid with both hypoxanthine and cyanide for the active site suggests that the metal mercaptide groups and the isoalloxazine ring must be located in close proximity

Fridovich and Handler [54] have proposed a structure for the active site of xanthine oxidase (Fig 22 3) The active site contains both FAD moieties only one of which is oxidizable by oxygen or dyes The metals at the active site are involved in several bonds i e to an adjacent sulfhydryl and to each of the flavins Electrons pass from the purine substrates to the first flavin and thence via the metal (ferric?) [55]

## XANTHINE OXIDASE

Xanthine oxidase is one of the most extensively studied enzymes. Its distribution in mammalian and avian tissues varies from species to species. In man xanthine oxidase is found in abundance in liver and small intestinal mucosa [41, 42] and possibly in bone marrow [42]. It has also been found in human milk [41] but all other human tissues studied have failed to show xanthine oxidase activity. These include blood, kidney, spleen, heart and skeletal muscle, lung and pancreas [41, 42].

Human liver xanthine oxidase has a substrate specificity identical with that of the bovine milk enzyme [33]. This observation implies that information derived from studies of the latter enzyme are relevant to man and this is fortunate since the great majority of careful studies have been conducted with bovine milk xanthine oxidase.

Xanthine oxidase is a flavoprotein containing molybdenum and iron [43]. Bovine milk xanthine oxidase contains two molecules of flavin adenine dinucleotide, one atom of molybdenum, and eight atoms of iron per molecule of enzyme and has a minimal molecular weight of approximately 320 000 [44]. Different FAD/Mo/Fe ratios have been reported for the enzyme obtained from mammalian intestine and liver and avian liver [45] but no observations are available on the human enzyme. The enzyme has a remarkably large number of substrates including (1) purines, (2) aldehydes and (3) pteridines. There is now some doubt that oxidation of DPNH by xanthine oxidase preparations is attributable to xanthine oxidase itself [46].

Purine compounds are oxidized in the 2, 6, or 8 position in the presence of xanthine oxidase. The order in which the oxidizable positions are attacked by xanthine oxidase varies from substrate to substrate. With purine the initial attack is at carbon 6. With hypoxanthine, the next attack is directed towards carbon 2. Positions 2 and 8 form a closely related pair, and oxidation at one of these carbons leads always to attack at the other partner in the next step [33].

Table 22.1 gives data on relative rates of oxidation of purine derivatives by bovine milk and human liver xanthine oxidases. In addition to the substrates shown, xanthine oxidase attacks adenine [47], 2-hydroxyadenine, 8-hydroxyadenine [48], 2,6-diaminopurine [49], 2-azaadenine, 2-azahypoxanthine [50], and a variety of other substituted purines. It does not attack methylxanthines other than the 1-methyl compound [33]. A variety of purine compounds is adsorbed by the enzyme without being oxidized [51]. The enzyme is competitively inhibited by a number of purine bases [49].

**Other Substrates.** All 31 different aldehydes which have been tested are oxidized to the corresponding acid by xanthine oxidase, although at

of an increment in xanthine output. If it is much less than 10 ml per min the calculated plasma level would soon be so high as to be inconsistent with experimental data already cited. Even a total inability to reabsorb xanthine in the renal tubule will therefore not explain excretion of xanthine at a level of 600 mg per gm creatinine.

*Metabolic Block Mechanism*

According to this hypothesis the xanthinuric patient lacks almost entirely the enzyme xanthine oxidase. Such a lack would no doubt lead to an accumulation of xanthine derived from guanine by action of guanase and possibly from xanthine nucleosides by action of nucleoside phosphorylase. The small amount of uric acid in urine might be attributed to dietary sources, to a remaining trace of activity of xanthine oxidase, or possibly to an accessory pathway such as cleavage of uric acid riboside [56]. The increase in hypoxanthine and of uric acid in urine on standing possibly represents a dismutation reaction



$$2\ \text{Xanthine} \longrightarrow \text{hypoxanthine} + \text{uric acid}$$

If xanthine oxidase were lacking one would anticipate an appreciable accumulation of hypoxanthine as well as of xanthine in plasma and urine, for there is good evidence that free hypoxanthine is normally an important intermediate in uric acid formation and the major oxypurine base normally present in plasma. Several possible mechanisms may be considered to explain a lack of hypoxanthine accumulation, all quite theoretical. (1) A hypoxanthine oxidase distinct from xanthine oxidase may exist. Such an enzyme has been postulated [57] but never identified. (2) Reutilization of hypoxanthine for nucleoside and nucleotide formation may be so efficient as to keep levels from rising abnormally. (3) Xanthine oxidase may not actually be missing but may be structurally abnormal; it may be able to bind and oxidize hypoxanthine but not able to oxidize xanthine.

The last possibility is suggested by recent evidence that the two-step oxidation of hypoxanthine to uric acid involves release of the intermediate xanthine from the enzyme surface and rebinding of xanthine, possibly to different active centers of the first FAD component. Bergmann and Dikstein [58] have suggested that hypoxanthine is bound at N1, N3 and N7, while xanthine is bound at N3, N7 and N9. An inability of the enzyme to bind the purine ring at N9 might render it incapable of acting upon xanthine without interfering with its action upon hypoxanthine. Or alternatively, such an enzyme abnormality might permit only very slow oxidation of xanthine, with production of only very small amounts of uric acid. If opportunity ever permits examination of liver or of breast milk of a xanthinuric subject for xanthine oxidase activity upon various substrates, this possibility would be of great interest to explore.

mercaptide groups which are each single electron acceptors to the second flavin from which they may pass to oxygen or other acceptors Mercury and cyanide both inhibit by interfering with the ability of the metal mercaptide group to transfer electrons from the first to the second flavin

This hypothesis would explain the occurrence of purine binding by the cyanide-inhibited enzyme and inhibition by high substrate concentration of purines which would also combine with the isoalloxazine ring of the

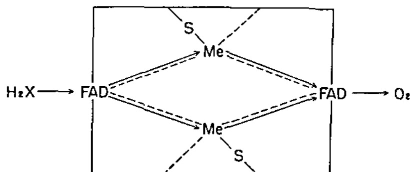



Fig 22-3 Proposed structure for the active site of xanthine oxidase (*Modified from Fridovich and Handler [54] with permission of the authors*)

autoxidizable flavin and thus prevent interaction with oxygen or other electron acceptors

## METABOLIC DEFECTS IN XANTHINURIA

Dent and Philpot [*1*] suggested that gross xanthinuria with very low levels of uric acid in both plasma and urine might be a consequence either of a block in oxidation of xanthine to uric acid or of a deviation' mechanism by which xanthine is excreted in urine too rapidly to allow much of it to be oxidized to uric acid Dickinson and Smellie [*15a*] have suggested that both defects are present and have published studies showing a renal clearance of oxypurines approaching that of the glomerular filtration rate in the original patient of Dent and Philpot

### *Renal Deviation Mechanism*

The deviation mechanism is not adequate as a single defect to explain the known findings In a normal adult with a glomerular filtration rate of 120 ml per min an excretion of xanthine of 6 mg per day and a xanthine clearance of 20 ml per min the plasma xanthine concentration would be of the order of 0 02 mg per 100 ml A person with a similar filtration rate and a total inability to reabsorb xanthine would then excrete only 36 mg xanthine per day and less if the plasma level declined as a consequence of renal xanthine loss If the normal clearance of xanthine is greater than 20 ml per min loss of ability to reabsorb xanthine would lead to even less

doubt add greatly to the burden of xanthine excretion and should be limited Methylxanthines such as caffeine and theophylline are very much more soluble than xanthine and are not metabolized by xanthine oxidase There would seem to be no unique reason to prohibit their use in xanthinuric subjects

## SUMMARY

1 Xanthinuria is a rare disorder characterized by the excretion of very large amounts of xanthine in urine and a tendency to form xanthine stones There is also a striking diminution of uric acid in serum and urine

2 In only one case that of a girl studied at 4½ and again at 9 years of age have adequate studies been performed to document xanthinuria and hypouricaciduria Several additional patients have formed xanthine stones in childhood and may provisionally be considered to have xanthinuria

3 Many of the adults who have formed xanthine stones have clearly not had the defect of the xanthinuric child for in several instances normal or elevated serum uric acid levels were found Circumstantial evidence suggests that xanthine excretion may have been excessive in a few but this is unproved In one case xanthine deposits were found in the renal parenchyma

4 The metabolic defect in xanthinuria probably involves both an absence of (or at least a defect in function of) xanthine oxidase coupled with a renal tubular defect in reabsorption of xanthine

5 The genetic factors in xanthinuria are wholly obscure except that an autosomal dominant inheritance is precluded

## BIBLIOGRAPHY


1 Dent C E and Philpot G R Xanthinuria an inborn error (or deviation) of metabolism Lancet 1 182 1954

2 Marcet A *An Essay on the Chemical History and Medical Treatment of Calculous Disorders* London 1817

3 Kretschmer H L Xanthin calculi Report of a case and a review of the literature J Urol 38 183 1937

4 Ratner M and Stra[illegible]berg A A case of xanthine calculi Canad M A J 40 350 1939

5 Hyman A and Leiter H E A case of xanthine calculi J Mt Sinai Hosp 8 81 1941

6 Butt A J and Hollman H D Jr Xanthine calculus a case report J Urol 52 89 1944

7 Gersh I J and Meltzer H L Xanthine urinary calculi two cases J Urol 55 169 1946

8 Berman L S Twenty-second case of xanthine urinary calculus J Urol 60 470 1948

9 Pearlman C K Xanthine urinary calculus J Urol 64 [illegible]9 1950

10 Taylor W N and Taylor J N Xanthine calculus case report J Urol 68 653 19[illegible]

11 Mackey J F Jr Xanthine calculus Missouri Med 50 617 1953

*A Combined Defect*

The studies of Dickinson and Smellie strongly suggest the presence of two defects, namely absence of xanthine oxidase and absence of the renal tubular absorptive mechanism for xanthine. Since human kidney does not contain xanthine oxidase activity [41, 42] these authors postulate that a single genetic defect has led to a defect in the synthesis of a specific protein serving as the apoenzyme of two catalysts having different physiologic roles. Alternatively, the results raise a question basic to biochemical genetics: does a gene (or genome) control the synthesis of a polypeptide fragment which can serve as a component of two or more larger polypeptide (protein) molecules?

## GENETICS

Thirteen relatives of the xanthinuric subject examined by Dent and Philpot [1] showed no abnormal excretion of xanthine and had normal outputs of uric acid. These included an only sister, both parents and three surviving grandparents. None gave a history of renal stone and the parents were not related. No statement regarding genetic factors other than exclusion of an autosomal dominant inheritance is justified at present.

In no instance has more than one case of xanthine stone formation been recorded in a single family. Both sexes have been affected, the sex distribution being 5 males and 1 female under age 15 and about 3 : 1 in favor of males in adults. Therefore no statement can be made regarding genetic factors involved in xanthine stone formation in general. Indeed the adult cases described may not be dependent upon inherited metabolic factors since there is presently no compelling evidence that a metabolic derangement exists in many such patients.

## TREATMENT

Prevention of xanthine stone formation in predisposed individual depends upon recognition of the low solubility of xanthine in acid solutions and of the possible tendency of certain of the affected individuals to excrete a highly acid urine. Dent and Philpot [1] found that 100 ml normal urine at 26 C dissolved 6.7, 6.5 and 16.5 mg xanthine at a pH of 5.8, 7.0 and 8.1, respectively. The $pK$ of xanthine is 7.7, the $pK$ is 10.6 [32].

A high fluid intake and oral alkali in quantities sufficient to maintain an alkaline urine would appear essential. Dietary regulation of purine intake is not indicated in the patient with a normal xanthine output for xanthine excretion is independent of diet in such patients [16]. However in a patient with a block in xanthine oxidation dietary purines would no

37 Jorgensen S Hypoxanthine and xanthine accumulated in stored human blood determination of relative amounts by spectrophotometry Acta pharmacol et toxicol 11 265 1955
38 Gutman A B Yu T F and Weissmann B The concept of secondary gout relation to purine metabolism in polycythemia and myeloid metaplasia Trans A Am Physicians 69 229 1956
39 Yu T F Weissmann B Sharney L Kupfer S and Gutman A B On the biosynthesis of uric acid from glycine $N^{15}$ in primary and secondary polycythemia Am J Med 21 901 1956
40 Gjorup S and Poulsen H Effects of probenecid cinchophen and colchicine on the plasma concentrations and renal excretion of oxypurine in patients with gout Acta pharmacol et toxicol 11 343 1955
41 Morgan E J The distribution of xanthine oxidase I Biochem J 20 1282 1926
42 Dunn J T and Wyngaarden J B Unpublished data
43 De Renzo E C Chemistry and biochemistry of xanthine oxidase Advances Enzymol 17 93 1956
44 Richert D A and Westerfeld W W The relationship of iron to xanthine oxidase J Biol Chem 209 179 154
45 Mehler A H Introduction to Enzymology p 1,8 Academic Press Inc New York 1957
46 Avis P G Bergel F and Bray R C Cellular constituents The chemistry of xanthine oxidase Part III Estimations of the co factors and the catalytic activities of enzyme fractions from cow s milk J Chem Soc p 1219 1956
47 Klenow H Adenine oxidase Biochem J 50 404 1951 52
48 Wyngaarden J B and Dunn J T 8-Hydroxyadenine as the intermediate in the oxidation of adenine to 2 8-dihydroxyadenine by xanthine oxidase Arch Biochem 70 150 1957
49 Wyngaarden J B 2 6-Diaminopurine as substrate and inhibitor of xanthine oxidase J Biol Chem 224 453 1957
50 Shaw E and Wolley D W Imidazo-1 2 3-triazines as substrates and inhibitors for xanthine oxidase J Biol Chem 194 641 195
51 Coombs H I Studies on xanthine oxidase IX The specificity of the system II Biochem J 21 1259 1927
52 Booth V H The specificity of xanthine oxidase Biochem J 32 494 1938
53 Kalckar H M Kjeldgaard N O and Klenow H Inhibition of xanthine oxidase and related enzymes by 6-pteridyl aldehyde J Biol Chem 174 771 1948
54 Fridovich I and Handler P Xanthine oxidase II Studies of the active site J Biol Chem 231 899 1958
55 Fridovich I and Handler P Xanthine oxidase IV Participation of iron in internal electron transport J Biol Chem 233 1581 1958
56 Laster L and Blair A Phosphorolytic cleavage of uric acid riboside by mammalian enzymes Fed Proc 17 261 1958
7 Buchanan J M in discussion by F Bergel R C Bray A Haddow and I Lewin Enzymatic control of purines by xanthine oxidase in *C ba Fo dation Sy iposi m on the Ch t y and B ology f Purines* ed ted by G E W Wolstenholme and C M O Connor p 267 Little Brown Boston 1957

12 Ichikawa T Xanthine calculi of kidney J Urol 72 770 1954
13 Jordan H Multiple Xanthinsteinbildung Bericht über einen Fall Deutsche Ztschr Verdauungskr 15 143 1955
14 Williams J N Jr Micromethod for determination of xanthine and guanine in urine J Biol Chem 184 627 1950
15 Folin O and Trimble H A system of blood analysis Supplement V Improvements in the quality and method of preparing the uric acid reagent J Biol Chem 60 473 1924
15a Dickinson C J and Smellie J M Xanthinuria Brit M J Dec 5 1217 1959
16 Weissmann B Bromberg P A and Gutman A B The purine bases of human urine I Separation and identification J Biol Chem 224 407 1957
17 Henneman P H Wallach S and Dempsey E F The metabolic defect responsible for uric acid renal stone formation J Clin Invest 37 901 1958
18 Orstrom A and Orstrom M The role of glutamine in metabolism with special regard to the formation of uric acid in gout Acta med scandinav 138 108 1950
19 Segal S and Wyngaarden J B Plasma glutamine and oxypurine contents in patients with gout Proc Soc Exper Biol & Med 88 342 1955
20 Friedman M and Byers S O Observations concerning the causes of excess excretion of uric acid in the Dalmatian dog J Biol Chem 175 127 1948
21 Praetorius E and Kirk J E Hypouricemia with evidence for tubular elimination of uric acid J Lab & clin Med 35 865 1950
22 Jorgensen S and Poulsen H E Enzymic determination of hypoxanthine and xanthine in human plasma and urine Acta pharmacol et toxicol 11 223 1955
23 Hitchings G H The estimation of guanine and xanthine J Biol Chem 139 843 1941
24 Beaver G H Holiday E R and Johnson E A Optical properties of nucleic acids and their components in *The Nucleic Acids Chemistry and Biology* edited by E Chargaff and J N Davidson vol 1 p 493 Academic Press Inc New York 1955
25 Kalckar H M Differential spectrophotometry of purine compounds by means of specific enzymes I Determination of hydroxypurine compounds J Biol Chem 167 429 1947
26 Krebs E G and Norris E R The competitive inhibition of xanthine oxidation by xanthopterin Arch Biochem 24 49 1949
27 Praetorius E An enzymatic method for the determination of uric acid by ultraviolet spectrophotometry Scandinav J Clin & Lab Invest 1 222 1949
28 Wyatt G R Separation of nucleic acid components by chromatography on filter paper in *The Nucleic Acids Chemistry and Biology* edited by E Chargaff and J N Davidson vol 1 p 243 Academic Press Inc New York 1955
29 Wyngaarden J B Blair A E and Hilley L On the mechanism of overproduction of uric acid in patients with primary gout J Clin Invest 37 579 1958
30 Weissmann B Bromberg P A and Gutman A B The purine bases of human urine II Semiquantitative estimation and isotope incorporation J Biol Chem 224 423 1957
31 Wyngaarden J B Seegmiller J E Laster L and Blair A E The utilization of hypoxanthine adenine and 4-amino 5-imidazole-carboxamide for uric acid synthesis in man Metabolism 8 455 1959
32 Bergmann F and Dikstein S The relationship between spectral shifts and structural changes in uric acids and related compounds J Am Chem Soc 77 691 1955
33 Bergmann F and Dikstein S Studies on uric acid and related compounds III Observations on the specificity of mammalian xanthine oxidases J Biol Chem 223 765 1956
34 Jorgensen S Xanthine formation from guanine guanosine or xanthosine in human blood Acta pharmacol et toxicol 12 303 1956
35 Greenstein J P Carter C E Chalkley H W and Leuthardt F M Enzymatic desamination and dephosphorylation of ribosenucleic and desoxyribosenucleic acids J Nat Cancer Inst 7 9 1946
36 Wyngaarden J B Unpublished data

literature has not revealed any report of the normal urinary excretion of orotic acid Examination of the urine of 73 human being both normal and with a variety of hematologic diseases has failed to reveal the presence of the compound with the single exception of the patient who provides the basis for this chapter [1] More extensive studies with more sensitive methods may modify the generality of this finding

*Biosynthesis of Orotic Acid*

The investigations elucidating the biosynthetic pathways leading to orotic acid and its role in the biosynthesis of pyrimidines have been adequately and recently reviewed [6-8] and all the details need not be repeated here The salient features of what now appears to be the major pathway of pyrimidine biosynthesis *de novo* are summarized in Fig 23 1 Several alternative pathways have been shown to exist but at present these seem to be of relatively minor quantitative importance in mammalian tissues

The first step shown in Fig 23 1 the carbamylphosphate synthetase reaction is not unique to orotic acid biosynthesis it also participates in the synthesis of citrulline from ornithine An active intermediate not yet unequivocally identified is involved [9] In bacteria the over all reaction is reversible but in mammals only the portion after the formation of the active intermediates has been demonstrated to be reversible [10] The next step the carbamylphosphate transferase reaction appears at present to be unique to orotic acid biosynthesis The equilibrium of this reaction lies so far towards carbamylaspartic acid [11] that it is essentially irreversible Ring closure in carbamylaspartic acid to yield dihydroorotic acid is effected by the enzyme dihydroorotase and in this instance the equilibrium lies about 2 1 towards carbamylaspartic acid [12] The oxidation of dihydroorotic acid is carried out by the DPN linked dihydroorotic dehydrogenase with the equilibrium lying far towards the left [13] It is to be noted despite these equilibrium points to the left that the probable presence of systems for the removal of DPNH would favor the accumulation of orotic acid if its further transformations were blocked This is precisely what happens in some bacteria [14] in *Neurospora* [15] and presumably in the patient who is the subject of this chapter [1]

*Biosynthesis of Pyrimidine Nucleotides*

Normally orotic acid is converted to the ribonucleotide orotidylic acid by reacting with 5 phosphoribosylpyrophosphate under the influence of the specific orotidylic pyrophosphorylase [16] This reaction is reversible with the equilibrium being about 9 1 in the direction of orotic acid Orotidylic acid is then decarboxylated to uridylic acid a reaction for which reversibility could not be demonstrated [16] Uridylic acid may then be converted to other pyrimidine nucleotides as indicated in the

# Chapter 23

## Oroticaciduria*

*Charles M. Huguley, Jr. and James A. Bain*

This chapter describes an abnormality thus far noted only once [1]. It occurred in the child of a consanguineous marriage. It is included in this book because it probably represents a genetically determined disorder of pyrimidine metabolism.

This child developed a severe anemia at age 5 months, characterized by hypochromic erythrocytes and megaloblastic marrow. The disease did not respond to treatment with iron, pyridoxine, vitamin $B_{12}$, folic acid, or ascorbic acid. The most intriguing feature was the passage in the urine of large amounts of crystals which proved to be orotic acid. There was some improvement in all features of the disease after the administration of adrenocorticoids and almost complete clearing of the evidence of illness after administration of a mixture of uridylic and cytidylic acid.

## OROTIC ACID

Orotic acid, 4-carboxyuracil, was first discovered in cow's milk [2] and has since been isolated from the milk of several species including man [3]. Even though it is an important intermediate in pyrimidine biosynthesis, orotic acid apparently does not accumulate in any appreciable concentration in animal tissues, since injected $C^{14}$ labeled orotate does not encounter any perceptible metabolic pools in rat liver or blood and the radioactive orotate disappears as such from the liver within 30 min [4]. The injected radioactive orotate is excreted with unchanged specific activity, and excretion is complete within 2 hr. Using isotope dilution techniques, Smith and Baker [5] were unable to find evidence of orotic acid in plasma, erythrocytes, or leukocytes in normal man. A search of the

* This work was supported by grants from the American Cancer Society (E-1R-13J) and from the John A. Hartford Foundation.

understood as a result of studies such as those from the laboratories of Kornberg [17] Ochoa [18] Kennedy [19] Leloir [20] and many others

The figure shows the central position of orotic acid an obligatory intermediate in pyrimidine synthesis *de novo* Pyrimidine bases derived from other endogenous sources or from exogenous sources may however be utilized for the formation of more complex molecules such as nucleic acid For example Cannellakis [21] has shown that uracil may be incorporated into ribonucleic acid at rates comparable to those of orotic acid incorporation when the conditions are such that a favorable competition exists between degradative and anabolic pathways Uracil and thymine have also been shown to react in liver preparations with deoxyribose 1 phosphate to yield the corresponding nucleosides [22]

Two pathways for the formation of thymidine and its phosphorylated derivatives are known (1) the better established one proceeds from uracil deoxyribonucleotide (or deoxyribonucleoside) by direct methylation to thymidylic acid (or thymidine) (2) the other proceeds from cytosine deoxyribonucleoside (or deoxyribonucleotide) either by methylation and subsequent deamination to the thymine derivatives or by deamination back through the uracil deoxyribonucleoside pathway The relative importance of these alternative pathways is yet to be completely evaluated for any mammalian tissue but the second is rendered doubtful by the work of Kit and associates [23]

## MEGALOBLASTOSIS

### *The Roles of Vitamin $B_{12}$ and Folic Acid*

The established hematopoietic efficacy of vitamin $B_{12}$ and folic acid in various types of megaloblastic anemia makes it appropriate here to consider briefly what is known of their biochemical roles

Evidence has been advanced indicating that vitamin $B_{12}$ may play a role in protein synthesis deoxyribose synthesis reduction of sulfhydryl groups methyl group synthesis and conversion of folic acid to an active form [24–31] Nevertheless despite the obvious importance of this vitamin its exact roles are not clearly delineated

The biochemical actions of folic acid while still not completely clear are much better known than are those of vitamin $B_{12}$ Folic acid is primarily involved in the transformation or transfer of one-carbon units in a variety of metabolic processes concerned with purines pyrimidines amino acids and probably other metabolites as well [24 25] It seems appropriate to consider here only its direct connection with pyrimidine biosynthesis which occurs at the conversion of deoxyuridylic acid to thymidylic acid The mechanism of this reaction has been postulated as a hydroxymethylation or formylation of deoxyuridylic acid through the intervention of the appropriate form of tetrahydrofolic acid (either

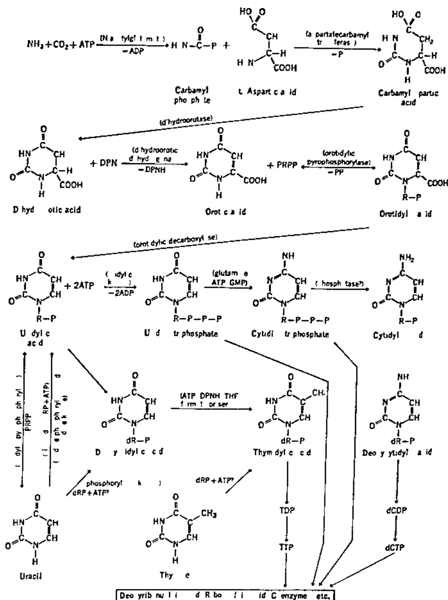



Fig 23 1 Biosynthesis of orotic acid and pyrimidine nucleotides

figure Much remains to be learned regarding these pathways implicit reliance cannot be placed in the generalizations of the figure

Figure 23 1 carries the pyrimidine transformations only as far as the nucleotide level The ultimate incorporation of the pyrimidine nucleotides into deoxyribonucleic acid ribonucleic acid the corresponding nucleoproteins and various coenzymes occurs by processes just beginning to be

cytoplasm and nucleus of the cell The resulting cell is gigantic and the distribution of DNA in the nucleus is sparse and delicate The cell matures with infrequent mitoses and the basophilic RNA remains in high concentration in the cytoplasm

Other information of particular relevance is the effect of administration of various pyrimidines and purines to patients with megaloblastic anemias The purines have been ineffective [39] Orotic acid [42] uracil [43] thymine [44] thymidine [45–49] and a mixture of uridylic and cytidylic acids [42] have all been effective in some cases though not in all The effects may be incomplete and are usually short lived In patients who have responded the dose of orotic acid has been 3 to 6 gm per day that of uracil 15 to 30 gm daily and that of thymine 10 to 15 gm daily These ratios are in keeping with what might be inferred from the previous discussion of pyrimidine biosynthesis Of particular interest are two patients [43 50 51] with megaloblastic anemia requiring folic acid who responded to 15 gm daily of thymine after failing to respond to 30 gm daily of uracil This seems to fit with the demonstrated need for folic acid in the methylation of uridylic acid to thymidylic acid

The previous discussion presents a basis for understanding the effects of these metabolites in folic acid deficiency but because the exact site or sites of vitamin $B_{12}$ activity is unknown there is no explanation for the effectiveness of these metabolites in vitamin $B_{12}$ deficiency One would hardly expect that any of these materials given singly would afford a universal or complete relief of the metabolic defects resulting from deficiency of either vitamin $B_{12}$ or folic acid Indeed it is remarkable how much some patients have improved and how long they have been maintained in remission while receiving only one of these metabolites

It is not to be assumed that the derangement in nucleic acid metabolism leading to formation of a megaloblastic marrow results only from absolute or relative deficiency of folic acid or vitamin $B_{12}$ It must be remembered that at each step in the metabolic chain an enzyme is necessary as well as the coenzyme (the vitamin) A genetic lack of an enzyme at any point in this metabolic chain (Fig 23 1) would be expected to produce a megaloblastic anemia It is only recently that the relative amount of such enzymes present in normal human cells has been investigated [5] Enzyme deficiency could explain several apparently inexplicable cases of megaloblastic anemia which have been reported including the one detailed below

## CLINICAL FEATURES OF OROTICACIDURIA

Full details of the clinical course of the only case of this abnormality so far reported have been given elsewhere [1] This child had a normal delivery at full term and no abnormalities were discovered at birth His health and development seemed normal until the age of 3 months From

hydroxymethyl or formyl) accompanied or followed by reduction to a methyl group with perhaps either DPNH or tetrahydrofolic acid acting as the reducing agent [32-34]. In considering the over all etiology of megaloblastic anemia it must be realized that folic acid like vitamin $B_{12}$ has manifold points of action in the metabolic sequences of the cell; it is not yet possible to say whether or not there is one point which is more critical than others for the normal development of the erythrocyte and other rapidly proliferating cells.

*Significance of Megaloblastosis*

The outstanding hematologic abnormality in the condition under discussion is the presence of megaloblasts in the marrow. This morphologic abnormality was originally described by Ehrlich [35] in the erythrocyte precursors. Strictly speaking the term *megaloblast* refers to an abnormal nucleated red blood cell. Similar changes however are observed in the granulocytes and megakaryocytes of the marrow in pernicious anemia [36] and in the epithelial cells of the gastrointestinal tract in the same disease [37].

The abnormality is a gigantism of the cell. The nucleus is large and frequently irregular and has a delicate chromatin pattern and large nucleoli. Basophilia of the cytoplasm persists very late as the cell matures. Reisner [38] has written a critical review of the nature and significance of megaloblastic blood formation.

The occurrence of a megaloblastic marrow is usually due to a deficiency of vitamin $B_{12}$ or folic acid and in nearly all instances will respond to the administration of one or both of these vitamins. Deficiency may result from dietary lack or from one of several conditions interfering with absorption. Megaloblastosis may also be seen occasionally in circumstances in which the mechanism is not fully understood but is believed to be due in part at least to increased requirements of one or the other of these vitamins [38]. It can also be produced in man by the administration of anti-folic acid or antipurine drugs and in susceptible individuals by the administration of diphenylhydantoin or primidone.

A few cases of megaloblastic anemia have responded to neither vitamin [39, 40]. Crystalluria was not described in these cases.

A second approach to the interpretation of megaloblastic changes is that of Thorell [41] who studied the DNA and RNA content of individual megaloblasts at different stages of development. Reisner [38] has reviewed more recent developments. The quantity of DNA in the nucleus is normal but large amounts of RNA persist in the cytoplasm to a very late stage in the development of the cell. It is theorized that the inability of the cell to synthesize DNA rapidly interferes with mitosis. The cell continues to grow and although the rate of production of RNA may also be subnormal large amounts of RNA are gradually deposited in the

At that time or subsequently a variety of diagnostic studies was made These studies as well as the patient s failure to respond to specific therapy, eliminated a deficiency of vitamin $B_{12}$ folic acid pyridoxine or iron as the cause of his anemia Hemoglobinopathy Mediterranean syndrome and Di Guglielmo s syndrome (erythremic myelosis) were also excluded

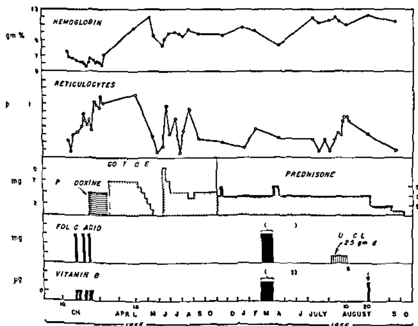



Fig 23-3 The lack of effect of parenteral folic acid vitamin B and pyridoxine and the partial beneficial effect of corticoids on hemoglobin and reticulocytes in a patient with oroticaciduria

The course of the disease is detailed in Figs 23 3 and 23-4 Figure 23 3 shows the failure of response to parenteral folic acid vitamin $B_{12}$ and pyridoxine Cortisone induced a partial hematologic remission which was later maintained by prednisone or prednisolone The marrow abnormalities were affected little if at all

Throughout the course orotic acid was present in the urine whenever it was examined If a good fluid intake and urine output were maintained the freshly voided urine would contain no crystals but large amounts would precipitate on standing When the intake of fluids was reduced as always happened when the patient felt ill voiding would be infrequent and crystals would precipitate in the bladder On several occasions urethral obstruction developed and on one occasion right ureteral obstruction Fortunately the obstructions always cleared when fluid

then until the time of his death at 2 years and 9 months of age he was chronically ill with anemia repeated respiratory infections recurrent diarrhea and crystalluria Prior to hematologic study he had been placed on a low fat diet which controlled the diarrhea and was given medications which he continued to receive for the rest of his life These assured a satisfactory intake of iron ascorbic acid folic acid vitamin $B_{12}$ liver concentrate and other vitamins

All ancestors were English or Scottish as far as is known except for one (Fig 23 2), a foundling believed to be French in origin who was a

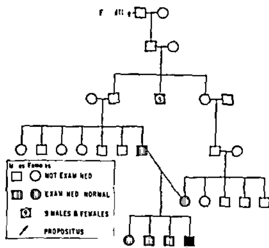



Fig 23 2 Pedigree of patient with oroticaciduria

common ancestor of both parents the great grandfather of the father and the great great-grandfather of the mother Neither parent nor any of three siblings had anemia abnormality of erythrocytes or oroticaciduria

When first studied at age 9 months the patient was pale and weak but well developed and apparently well nourished The sclerae were definitely blue There was torsion of the left tibia outward but no other skeletal abnormality The tongue had a normal coat The edge of the liver and the tip of the spleen were just palpable

A severe anemia was found The erythrocytes were hypochromic with a striking anisocytosis and poikilocytosis There were a few nucleated erythrocytes The reticulocyte percentage was slightly elevated There was a leukopenia with a monocytosis The platelet count was normal The marrow was hypercellular and had striking abnormalities of the megaloblastic type in cells of both the granulocytic and rubricytic series The urine was negative except for a moderate number of crystals which were later proved to be orotic acid

Prednisone was discontinued and the patient was permitted to relapse Figure 23-4 indicates the subsequent re ponse to prednisolone a moderate reticulocyte ri e and improvement in the hemoglobin level a rise in the white blood cell count and in the percentage of neutrophils There was a fall in the orotic acid excretion but the level remained high

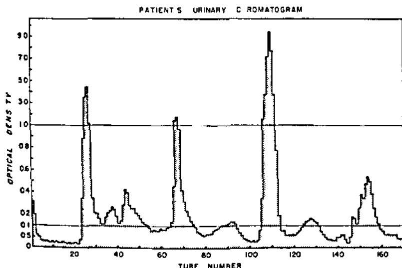



Fig 23 5 Column chromatogram of patient s urine Chromatograms of this type were obtained by applying a suitably diluted sample of urine adjusted to pH 8 0 containing approximately 150 to 200 optical density units measured at 260 mμ to a 1 × 10 cm Dowex 1-chloride column The column was washe l with twice the sample volume of water and eluted with HCl using a continuous grad ent elution system [5*] The 260-ml mixing flask contained water at the beginning 435 ml 0 015 N HCl 200 ml 0 1 N HCl and 100 ml 5 N HCl were run succe ively through the mixing flask from a reservoir under enough air pressure to give a rate of 0 5 to 0 7 ml p r min Then 100 ml 5 N HCl was run directly onto the column to remove any uneluted material Optical d n ity of each fraction was read on a Beckman DU spectrophotometer at 260 and 285 mμ but only the 260 mμ values are plotted in this figure The ratio 285 260 was used for rapid identificat on of the peaks Only two of the peaks gave ratios greater than 1 (1) that centered at tube No 6 is uric acid with a ratio of 2 6 (2) that centered at tube No 109 is orotic ac d with a ratio of 1 8

The addition of a mixture of nucleotides[1] effected an excellent reticulocyto is rise in the hemoglobin level to normal and a striking reduction in the excretion of orotic acid The appearance of the erythrocytes became normal and megalobla ts disappeared from the marrow The child gained weight for the first time in 18 months and lo t his apparent retardation in walking talking and general activity

[1] Con ent ated from yeast extract by Schwartz Laboratorie Inc Mt Vernon N Y conta ning 11.5 mg cyt dylic a i l an l 269 mg uridyl c acid per milliliter

intake was increased Proof of the identification of these crystals as orotic acid is given elsewhere [1] Briefly the excreted material corresponded to authentic orotic acid samples with respect to elution characteristics from ion-exchange columns $R_F$ values on paper chromatograms

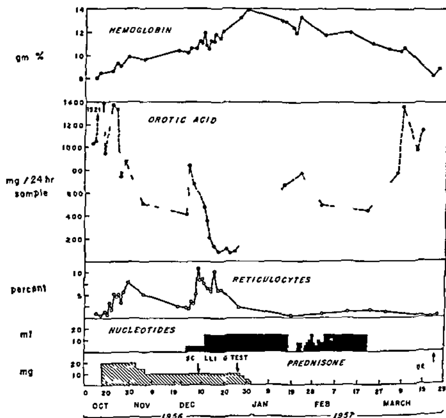



Fig 23-4 Changes in hemoglobin reticulocytes and urinary orotic acid in a patient with oroticaciduria as influenced by administration of prednisolone and a mixture of nucleotides Medication is diagrammed in the lowest two segments of the figure The nucleotide mixture contained uridylic acid 115 mg per ml and cytidylic acid 269 mg per ml

in several solvent systems ultraviolet absorption spectrums melting points of the original material and derivative and elemental analysis Determinations were made by fractionation on a Dowex column and ultraviolet *spectrophotometric* determination of the concentration in the appropriate fractions of eluate (Fig 23 5)

Figure 23 3 also shows the hematologic ineffectiveness of uracil An attempt was made to observe the effect of uracil on orotic acid excretion but the technique for collecting 24 hr urine specimens was inadequate at that time Orotic acid excretion was reduced little if at all

separate administration of uridylic and orotidylic acid which might have provided a more definitive localization of the defect

This case also provides an instructive illustration of the negative feed back mechanisms which control the production of intermediates in biosynthetic pathways by regulating either the formation of enzymes or their activity [53] Such feed back mechanisms have been shown in various microorganisms to control the formation of purine precursors [54] arginine [55] isoleucine [56] and orotic acid [14 57–59] among

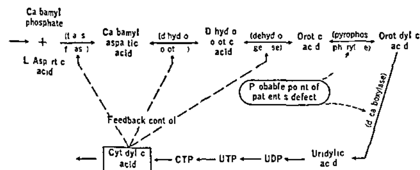



Fig 3 6 Probable point of metabolic defect and possible sites of feed back control mechanism

others Similar mechanisms have been inferred from experiments with avian [60] and mammalian systems [61 62] The case under discussion seems to be most closely analogous to that described in *Escherichia coli* mutants by Yates and Pardee [59] in which it was found that accumulation of carbamylaspartate dihydroorotate and orotate was inhibited by the presence of uracil or cytosine This inhibition was shown to be the result of the fact that cytidine and especially cytidylic acid are competitive inhibitors of aspartatecarbamyl transferase The mechanism of inhibition by the cytidylic uridylic acid mixture of orotic acid accumulation in the patient can only be inferred by analogy but it seems probable that a control mechanism existed in this patient similar to that described by Yates and Pardee in bacteria The situation may be summarized as in Fig 23-6

Aspartate carbamyl transferase appears to be the most probable point at which control is exerted This reaction is favored because it is the first one which is specific for pyrimidine biosynthesis and because it is irreversible Cytidylic acid is shown in the figure as the most likely controlling material because of its relative specificity as an inhibitor of the transferase as shown by the work of Yates and Pardee [58] It should be noted however that Smith and Baker [63] have reported that in contrast

When prednisolone was then stopped the remission continued but when nucleotides were stopped the patient rapidly relapsed and while in relapse contracted a severe case of varicella which involved the lungs and led to his death At autopsy no abnormalities were found which might have explained the megaloblastic anemia and oroticaciduria

As far as has been determined from a search of the literature this case is unique As indicated above it does not fall into any of the well-defined categories of megaloblastic anemia nor does the megaloblastosis seem to be a secondary phenomenon While cases of idiopathic vitamin $B_{12}$- and folic acid-refractory anemia have been described [39, 40] the authors have been unable to find a case on record of oroticaciduria or even a note of crystalluria as a feature of megaloblastic anemia

The recognition of crystalluria in this case was due to the combination of a rather massive excretion of orotic acid and a disinclination of the patient to take much liquid when ill In other circumstances it might easily have been missed Nevertheless the urines of 73 other persons have been negative for orotic acid These included specimens from normal children and adults, both parents and all three siblings of the patient 19 persons (some in relapse some after therapy) with various types of megaloblastic anemia including intestinal malabsorption syndrome nutritional megaloblastic anemia and the megaloblastic anemia due to primidone and diphenylhydantoin as well as Addisonian pernicious anemia three patients with erythremic myelosis, and patients with a variety of nonmegaloblastic anemias

## BIOCHEMICAL CONSIDERATIONS

The findings clearly imply a defect in the pyrimidine synthetic pathway *de novo* between orotic acid and uridylic acid (or perhaps cytidylic acid) The region of the block may be further delimited if it is assumed that in man the orotidylic decarboxylase reaction is irreversible, as it is in yeast [16] Since the equilibrium between orotic acid and orotidylic acid lies far towards the former a defect in either orotidylic pyrophosphorylase or *orotidylic decarboxylase would account for the observed accumulation of* orotic acid (Fig 23 6) There was at least a tenfold preponderance of the orotic acid peak in the urinary chromatogram over *the adjacent peaks* (Fig 23 5) and the absorption spectrums of the adjacent peaks showed little resemblance to those of suspected intermediates Unfortunately an exhaustive characterization of the minor components of the urinary chromatogram was not performed so that the excretion of small amounts of orotidylic acid or orotidine (which might arise from dephosphorylation of the former) cannot be ruled out with certainty In any case the excreted materials are probably not strictly relatable in quantity to those occurring at the tissue *of origin* The early death of the patient prevented the

## BIBLIOGRAPHY


1 Huguley C M Jr Bain J A Rivers S and Scoggins R Refractory megaloblastic anemia associated with excretion of orotic acid Blood 14 615 1959
2 Biscaro G and Belloni E Über die Orotsäure (chemisches Zentralblatt) Ann Soc Chim Milano 11 18 71 1905
3 Bendich A Chemistry of purines and pyrimidines in *The Nucleic Acids* edited by E Chargaff and J N Davidson vol I p 81 Academic Press Inc New York 1955
4 Hurlbert R B and Potter V Nucleotide metabolism conversion of orotic acid 6-C[14] to uridine nucleotides J Biol Chem 209 1 1954
5 Smith L H Jr and Baker F A Pyrimidine metabolism in man I The biosynthesis of orotic acid J Clin Invest 38 798 1959
6 Reichard P Biosynthesis of purines and pyrimidines in *The Nucleic Acid* edited by E Chargaff and J N Davidson vol II p 277 Academic Press Inc New York 1955
7 Carter C E Metabolism of purines and pyrimidines Ann Rev Biochem 25 1 3 1956
8 Heppel L A and Rabinowitz J C Enzymology of nucleic acids purines and pyrimidines Ann Rev Biochem 27 613 1958
9 Metzenberg R L Marshall M and Cohen P P Purification of carbamyl phosphate synthetase from frog liver J Biol Chem 233 102 1958
10 Metzenberg R L Marshall M and Cohen P P Carbamyl phosphate synthetase studies on the mechanism of action J Biol Chem 233 1560 1958
11 Reichard P and Hanshoff G Aspartate carbamyl transferase from *Escherichia coli* Acta chem scandinav 10 548 1956
12 Lieberman I and Kornberg A Enzymatic synthesis and breakdown of a pyrimidine orotic acid II Dihydroorotic acid ureidosuccinic acid and 5-carboxymethylhydantoin J Biol Chem 207 911 1954
13 Lieberman I and Kornberg A Enzymic synthesis and breakdown of a pyrimidine orotic acid I Dihydro-orotic dehydrogenase Biochim et biophys acta 12 223 1953
14 Brooks M S Ushiba D and Magasanik B Some factors affecting the excretion of orotic acid by mutants of *Aerobacter aerogenes* J Bact 68 534 1954
15 Mitchell H K Houlahan M B and Nyc J F The accumulation of orotic acid by a pyrimidineless mutant of *Neurospora* J Biol Chem 172 525 1948
16 Lieberman I Kornberg A and Simms E S Enzymatic synthesis of pyrimidine nucleotides Orotidine 5 phosphate and uridine-5 phosphate J Biol Chem 215 403 1955
17 Bessman M J Lehman I R Simms E S and Kornberg A Enzymatic synthesis of deoxyribonucleic acid II General properties of the reaction J Biol Chem 233 171 1958
18 Grunberg Manago M Ortiz P J and Ochoa S Enzymatic synthesis of polynucleotides I Polynucleotide phosphorylase of *Azotobacter vinelandii* Biochim et biophys acta 20 269 1956
19 Kennedy E P and Weiss S B The function of cytidine coenzymes in the biosynthesis of phospholipides J Biol Chem 222 193 1956
20 Cardini C E and Leloir L F Enzymatic formation of acetylgalactosamine J Biol Chem 225 317 1957
21 Canellakis E S Pyrimidine metabolism III The interaction of the catabolic and anabolic pathways of uracil metabolism J Biol Chem 227 701 195
22 Kornberg A Pathways of enzymatic synthesis of nucleotides and polynucleotides in *The Chemical Basis of Heredity* edited by W D McElroy and B Glass p 5 9 Johns Hopkins Press Baltimore 19
23 Kit S Beck C Graham O L and Gross A Further studies on the pathways of synthesis of thymine compounds by lymphatic tissues and tumors J Biol Chem 233 944 1958

to the bacterial enzyme the aspartate carbamyl transferase from human blood cells is not inhibited by cytidylic acid Obviously either dihydroorotase or the dehydrogenase might be the point of control, and uridylic acid UDP, UTP, CTP or other compounds derived from them are candidates for the controlling material Uridine diphosphate has been shown to control the activity of orotidylic decarboxylase [63] in a mammalian system Extension of such studies as these will probably allow an eventual evaluation of the relative effectiveness of the members of the pyrimidine biosynthetic system as inhibitors of the various enzymes involved

## GENETIC CONSIDERATIONS

The evidence that this disease is due to the deficiency of an enzyme has been given above Since symptoms appeared early in life there is strong presumption that the enzyme deficiency was genetic in origin but since no other members of the family are known to have been affected there is no proof that it is genetic Nevertheless because of the consanguinity of the parents the opportunity was present for the apparent transmission of a recessive trait in the homozygous state in the propositus (Fig 23 2) If this is what occurred the heterozygous state is not associated with oroticaciduria nor with anemia or morphologic abnormalities of the mature erythrocyte

## SUMMARY

1 Oroticaciduria has been described once It was associated with a severe anemia developing in infancy and was characterized by hypochromic erythrocytes and a megaloblastic marrow There was some improvement in all features of the disease after the administration of adrenocorticoids and almost complete clearing of the evidence of illness after the administration of a mixture of uridylic and cytidylic acids

2 Megaloblastosis would be expected as a result of failure in pyrimidine synthesis Relief by administration of uridylic and cytidylic acid is as might be predicted the subsequent fall in orotic acid excretion is probably attributable to a negative feed back control mechanism as has been demonstrated in bacterial systems

3 Orotic acid is an obligatory intermediate in *de novo* pyrimidine synthesis The excretion of large amounts would indicate a block in metabolic progression to orotidylic acid or to uridylic acid A lack of either orotidylic pyrophosphorylase or orotidylic decarboxylase would account for the observed accumulation of orotic acid

4 Recently reduced levels of both enzymes were found in the erythrocytes of both parents and two of the surviving siblings of the deceased propositus [64]

# BIBLIOGRAPHY


1 Huguley C M Jr Bain J A Rivers S and Scoggins R Refractory megaloblastic anemia associated with excretion of orotic acid Blood 14 615 1959
2 Biscaro G and Belloni E Über die Orotsaure (chemisches Zentralblatt) Ann Soc Chim Milano 11 18 71 1905
3 Bendich A Chemistry of purines and pyrimidines in *The Nucleic Acids* edited by E Chargaff and J N Davidson vol I p 81 Academic Press Inc New York 1955
4 Hurlbert R B and Potter V Nucleotide metabolism conversion of orotic acid 6-C to uridine nucleotides J Biol Chem 209 1 1954
5 Smith L H Jr and Baker F A Pyrimidine metabolism in man I The biosynthesis of orotic acid J Clin Invest 38 798 1959
6 Reichard P Biosynthesis of purines and pyrimidines in *The Nucleic Acids* edited by E Chargaff and J N Davidson vol II p 277 Academic Press Inc New York 1955
7 Carter C E Metabolism of purines and pyrimidines Ann Rev Biochem 25 123 1956
8 Heppel L A and Rabinowitz J C Enzymology of nucleic acids purines and pyrimidines Ann Rev Biochem 27 613 1958
9 Metzenberg R L Marshall M and Cohen P P Purification of carbamyl phosphate synthetase from frog liver J Biol Chem 233 10? 1958
10 Metzenberg R L Marshall M and Cohen P P Carbamyl phosphate synthetase studies on the mechanism of action J Biol Chem 233 1560 1958
11 Reichard P and Hanshoff G Aspartate carbamyl transferase from *Escherichia coli* Acta chem scandinav 10 548 1956
12 Lieberman I and Kornberg A Enzymatic synthesis and breakdown of a pyrimidine orotic acid II Dihydroorotic acid ureidosuccinic acid and 5-carboxymethylhydantoin J Biol Chem 207 911 1954
13 Lieberman I and Kornberg A Enzymic synthesis and breakdown of a pyrimidine orotic acid I Dihydro-orotic dehydrogenase Biochim et biophys acta 12 ? 3 1953
14 Brooks M S Ushiba D and Magasanik B Some factors affecting the excretion of orotic acid by mutants of *Aerobacter aerogenes* J Bact 68 534 1954
15 Mitchell H K Houlahan M B and Nyc J F The accumulation of orotic acid by a pyrimidineless mutant of *Neurospora* J Biol Chem 172 525 1948
16 Lieberman I Kornberg A and Simms E S Enzymatic synthesis of pyrimidine nucleotides Orotidine-5 phosphate and uridine 5 phosphate J Biol Chem 215 403 1955
17 Bessman M J Lehman I R Simms E S and Kornberg A Enzymatic synthesis of deoxyribonucleic acid II General properties of the reaction J Biol Chem 233 171 1958
18 Grunberg Manago M Ortiz P J and Ochoa S Enzymatic synthesis of polynucleotides I Polynucleotide phosphorylase of *Azotobacter vinelandii* Biochim et biophys acta 20 269 1956
19 Kennedy E P and Wei[illegible] S B The function of cytidine coenzymes in the biosynthesis of phospholipides J Biol Chem 222 193 1956
20 Cardini C E and Leloir L F Enzymatic formation of acetylgalactosamine J Biol Chem 226 317 1957
21 Canellakis E S Pyrimidine metabolism III The interaction of the catabolic and anabolic pathways of uracil metabolism J Biol Chem 227 ?01 195?
22 Kornberg A Pathways of enzymatic syntheses of nucleotides and polynucleotides in *The Chem cal Ba is f Her d ty* edite l by W D McElroy and B Glass p 5 9 Johns Hopkins Press Baltimore 19[illegible]
23 [illegible] S Beck C Graham O L and [illegible] A Further studies on the pathway of synthesis of thymine compounds by lymphatic tissues and tumors J Biol Chem 233 914 1958.

24 Broquist H P Water-soluble vitamins Part I (Folic acid $B_{12}$ group choline) Ann Rev Biochem 27 285 1958
25 Totter J R Water-soluble vitamins Part I (Vitamin $B_{12}$ folic acid choline and para aminobenzoic acid) Ann Rev Biochem 26 181 1957
26 Welch A D Nutritional role of folic acid Nutrition Rev 15 33 1957
27 Editorial Staff The metabolic role of vitamin $B_{12}$ Nutrition Rev 15 312 1957
28 Dinning J S Allen B K Young R S and Day P L The role of vitamin $B_{12}$ in thymine biosynthesis by *Lactobacillus leichmannii* J Biol Chem 233 674 1958
29 Wagle S R Vaughan D A Mistry S P and Johnson B C Vitamin $B_{12}$ and nucleic acid biosynthesis J Biol Chem 230 917 1958
30 Downing M and Schweigert B S Role of vitamin $B_{12}$ in nucleic acid metabolism IV Metabolism of C labeled thymidine by *Lactobacillus leichmannii* J Biol Chem 220 521 1956
31 Wagle S R Mehta R and Johnson B C Vitamin $B_{12}$ and protein biosynthesis VI Relation of vitamin $B_{12}$ to amino acid activation J Biol Chem 233 619 1958
32 Friedkin M and Kornberg A The enzymatic conversion of deoxyuridylic acid to thymidylic acid and the participation of tetrahydrofolic acid in *The Chemical Basis of Heredity* edited by W D McElroy and B Glass p 609 Johns Hopkins Press Baltimore 1957
33 Friedkin M The transfer of $H^3$ from tritiated tetrahydrofolic acid to thymidylic acid Fed Proc 18 230 1959 (abstract)
34 Humphreys G K and Greenberg D M Studies on the conversion of deoxyuridylic acid to thymidylic acid by a soluble extract from rat thymus Arch biochim et biophys 78 275 1958
35 Ehrlich P De Und Regeneration roter Blutscheiben Verhandl deutsch Gesellsch Chir June 10 and Dec 9 1880
36 Dameshek W and Valentine E H The sternal marrow in pernicious anemia Arch Path 23 159 1937
37 Graham R M and Rheault M H Characteristic cellular changes in epithelial cells in pernicious anemia J Lab & Clin Med 43 235 1954
38 Reisner E H Jr The nature and significance of megaloblastic blood formation Blood 13 313 1958
39 Vilter R W and Mueller J F The growth and maturation of the erythrocyte in *Monographs in Medicine* vol I p 519 Williams & Wilkins Baltimore 1952
40 Roelsen E and Ohlsen A S Achrestic anemia completely refractory megaloblastic anemia A case followed for nearly four years Acta med scandinav 150 17 1954
41 Thorell B Studies on the formation of cellular substances during blood cell production Acta med scandinav Suppl 200 1 1947
42 Rundles R W and Brewer S S Jr Hematologic responses in pernicious anemia to orotic acid Blood 13 99 1958
43 Vilter R W Horrigan D Mueller J F Jarrold T Vilter C F Hawkins V R and Seaman A Studies on relationship of vitamin $B_{12}$ folic acid thymine uracil and methyl group donors in persons with pernicious anemia and related megaloblastic anemias Blood 5 695 1950
44 Frommeyer W B Spies T D Vilter C F and English A Further observations on the antianemic properties of 5-methyl uracil J Lab & Clin Med 31 643 1946
45 Hausmann K Haemopoietic effect of thymidine in pernicious anemia Lancet 1 329 1951
46 Ungley C C Thymidine and vitamin $B_{12}$ in pernicious anemia Lancet 1 164, 1949
47 Reisner E H and West R Effect of thymine desoxyriboside (thymidine) on human pernicious anemia Proc Soc Exper Biol & Med 71 651 1949
48 Spray G H and Witts L J Thymidine in megaloblastic anemia Lancet 2 869 1958

49 Butterworth C E Jr and Perez Santiago E An evaluation of thymidine in treatment of tropical sprue Proc Soc Exper Biol & Med 92 762 1956
50 Mueller J F and Will J J Interrelationship of folic acid vitamin $B_{12}$ and ascorbic acid in patients with megaloblastic anemia Am J Clin Nutrition 3 30 1955
51 Mueller J F Hawkins V R and Vilter R W Liver extract in refractory megaloblastic anemia Blood 4 1117 1949
52 Hurlbert R B Schmitz H Brumm A F and Potter V R Nucleotide metabolism II Chromatographic separation of acid-soluble nucleotides J Biol Chem 209 23 1954
53 Pardee A B The control of enzyme activity in *The Enzymes* 2d ed edited by P D Boyer H Lardy and K Myrbach vol I p 681 Academic Press Inc New York 1959
54 Gots J S Purine metabolism in bacteria V Feedback inhibition J Biol Chem 228 57 1957
55 Gorini L and Maas W K The potential for the formation of a biosynthetic enzyme in *Escherichia coli* (Short communication) Biochim et biophys acta 25 208 1957
56 Umbarger H E and Brown B Isoleucine and valine metabolism in *Escherichia coli* VII A negative feedback mechanism controlling isoleucine biosynthesis J Biol Chem 233 415 1958
57 Yates R A and Pardee A B Control of pyrimidine biosynthesis in *Escherichia coli* by a feed back mechanism J Biol Chem 221 757 1956
58 Yates R A and Pardee A B Control of uracil of formation of enzymes required for orotate synthesis J Biol Chem 227 677 1957
59 Gorini L and Maas W K Feed back control of the formation of biosynthetic enzymes in *The Chemical Basis of Development* edited by W D McElroy and B Glass p 469 Johns Hopkins Press Baltimore 1958
60 Wyngaarden J B and Ashton D M The regulation of activity of phosphoribosylpyrophosphate amidotransferase by purine ribonucleotides a potential feedback control of purine biosynthesis J Biol Chem 234 1492 1959
61 Potter V R The biochemical approach to the cancer problem Fed Proc 17 691 1958
62 Potter V R and Blair D G R A negative feedback mechanism in pyrimidine biosynthesis Fed Proc 18 304 1959 (abstract)
63 Smith L H Jr and Baker F A Pyrimidine studies in pernicious anemia J Clin Invest 38 1044 1959 (abstract)
64 Smith L H Jr and Huguley C M Jr The enzymatic defect of orotic aciduria J Clin Invest 39 1029 1960 (abstract)

# Chapter 24

## Beta aminoisobutyricaciduria*

H Eldon Sutton

The development of paper chromatography provided a powerful tool for the recognition of hitherto undetected substances in biologic fluids Among the first to exploit the potentials of this technique was Dent, who examined urine samples from many normal and diseased persons and recorded the presence of several previously unrecognized substances which form colored products with ninhydrin [1] Of the substances that he encountered in a number of samples was one which he first designated as T spot and which was later identified independently by Crumpler et al [2] and by Fink Henderson and Fink [3] as β aminoisobutyric acid (α methyl β alanine) Crumpler et al [2] found that readily detectable amounts of β aminoisobutyric acid (BAIB) occurred persistently in the urine of approximately 4 8 per cent of normal British subjects They suggested that the rate of excretion is controlled by genetic factors On the other hand Fink et al [3] in addition to finding a small number of normal individuals who were high excretors found that a large percentage of cancer patients also were high excretors Figure 24 1 shows photographs of two-dimensional amino acid chromatograms one from a high BAIB excretor and the other from a low excretor

Since these early papers both the genetic basis for BAIB excretion and the association of BAIB excretion with certain types of disease have been amply confirmed Harris [4] studied a series of families in London and reported that some individuals consistently excrete large amounts of BAIB (70 to 300 mg per day) and that these persons tend to occur in the same families Other studies have confirmed this On the other hand BAIB excretion has also been reported in association with certain neoplastic diseases, particularly leukemia [3 5–10] and with tuberculosis [11] march hemoglobinuria [12] liver disease [13] lead poisoning [14]

Research in the author s laboratory is supported in part by Research Grant A 830 from the National Institutes of Health Public Health Service

mongolian idiocy and other types of mental defect [15–17] epilepsy [18] and following heavy irradiation with neutrons and $\gamma$ rays [19] Several other diseases have been reported with high BAIB excretion but in most cases these have involved too few patients to exclude a chance association with an inherited high excretion of BAIB or else the procedures for identifying BAIB were inadequate Berry [19a] has recently summarized studies of BAIB excretion in a large number of healthy and diseased

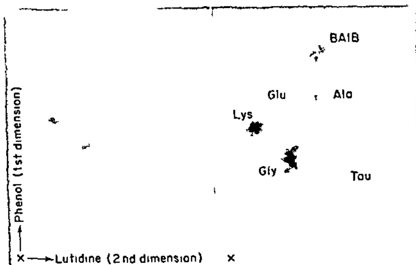



Fig 24 1 Two-dimensional amino acid chromatograms of urine from a low BAIB excretor (*left*) and from a high BAIB excretor (*right*) An aliquot of urine containing 50 µg creatinine was applied in the lower left corner Color development was with ninhydrin Abbreviations alanine (Ala) glutamine (Glu) glycine (Gly) lysine (Lys) and taurine (Tau)

infants She found that the high excretion associated with diseases is variable and that in some cases it could be influenced by diet No evidence has been obtained that inherited BAIB excretion is deleterious

Although BAIB frequently occurs in high levels in urine it does not occur in sufficient quantities for detection in other body fluids which have been tested (blood spinal fluid saliva) It was not found at appreciable levels in urine samples from 55 primates [10] On the other hand Tallan Moore and Stein [20] reported barely detectable quantities in the liver brain and kidneys of a cat Its occurrence in appreciable concentrations however seems limited to the urine of some human beings

## METABOLIC REACTIONS INVOLVING BAIB

### *Relation to Thymine*

In their paper on the identity of BAIB Fink Henderson and Fink [3] recognized that the carbon skeleton of BAIB is present in thymine and

# Chapter 24

## Beta aminoisobutyricaciduria*

H Eldon Sutton

The development of paper chromatography provided a powerful tool for the recognition of hitherto undetected substances in biologic fluids Among the first to exploit the potentials of this technique was Dent who examined urine samples from many normal and diseased persons and recorded the presence of several previously unrecognized substances which form colored products with ninhydrin [1] Of the substances that he encountered in a number of samples was one which he first designated as 'T' spot and which was later identified independently by Crumpler et al [2] and by Fink Henderson and Fink [3] as β aminoisobutyric acid (α methyl β alanine) Crumpler et al [2] found that readily detectable amounts of β aminoisobutyric acid (BAIB) occurred persistently in the urine of approximately 4 8 per cent of normal British subjects They suggested that the rate of excretion is controlled by genetic factors On the other hand Fink et al [3] in addition to finding a small number of normal individuals who were high excretors found that a large percentage of cancer patients also were high excretors Figure 24 1 shows photographs of two-dimensional amino acid chromatograms one from a high BAIB excretor and the other from a low excretor

Since these early papers both the genetic basis for BAIB excretion and the association of BAIB excretion with certain types of disease have been amply confirmed Harris [4] studied a series of families in London and reported that some individuals consistently excrete large amounts of BAIB (70 to 300 mg per day) and that these persons tend to occur in the same families Other studies have confirmed this On the other hand BAIB excretion has also been reported in association with certain neoplastic diseases particularly leukemia [3 5–10] and with tuberculosis [11] march hemoglobinuria [12] liver disease [13] lead poisoning [14],

Research in the author s laboratory is supported in part by Research Grant A 830 from the National Institutes of Health Public Health Service

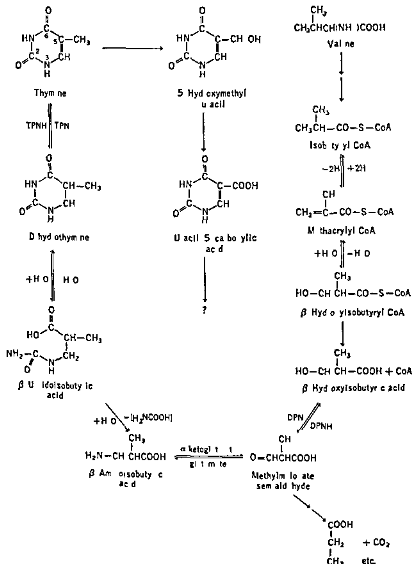



Fig 24.? Estal l le l m tal ol c reactior of BAIB ar l relate l sul tance

The same enzyme preparation (partially purified) also used β-alanine and γ aminobutyric acid as substrates

*Steric Considerations*

Since the α-carbon of BAIB is a ymmetric the question of optical isomeri m should be con idered in treating the metaboli m of BAIB

that BAIB might therefore be a metabolic end product of thymine Evidence favoring this hypothesis was obtained by feeding large amounts of DNA and RNA to rats BAIB cannot normally be detected in the urine of rats but on the DNA diet it is excreted By contrast the RNA diet which does not contain thymine does not cause the excretion of BAIB The high levels of nucleic acid used in these diets (40 per cent) raised doubts as to the significance of the effect observed but subsequent studies by this same group [21] have confirmed that BAIB can be a metabolite of thymine Of a variety of pyrimidines injected into rats only thymine and dihydrothymine caused the appearance of BAIB in the urine Even 5-methylcytosine which has the same carbon skeleton as thymine failed to cause the excretion of BAIB

Both thymine and dihydrothymine can yield BAIB when incubated with rat liver or kidney slices [22] Liver is more active in this respect Dihydrothymine is a much more effective precursor than thymine and thymidine is no better than thymine No BAIB is formed when these substrates are incubated with a variety of tissues other than liver and kidney On the other hand liver and kidney from a variety of mammals were shown to form BAIB

In a subsequent series of papers the Finks and their associates have demonstrated the conversion of thymine by way of dihydrothymine and $\beta$ ureidoisobutyric acid to BAIB as shown in Fig 24 2 [23 24] An analogous series of reactions was also demonstrated for the conversion of uracil to $\beta$ alanine [23] and there is evidence that the cleavage of the dihydropyrimidine ring is catalyzed by the same enzyme in both pathways [25] Canellakis [26] studied these reductive degradation pathways for uracil and thymine and reported that the initial reduction of the pyrimidine ring requires reduced TPN Grisolia and his associates [27 28] studied the decarbamylation reactions of $\beta$ ureidopropionic acid and $\beta$ ureidoisobutyric acid and found that formation of the $\beta$ amino acids is accompanied by the production of $NH_3$ and $CO_2$ However Fink et al [29] using labeled thymine found that the C2 carbon appears in urea In addition they found that some of the thymine is oxidized at the methyl group indicating an alternate oxidative pathway for thymine degradation Whether the urea is formed from oxidative degradation or from the carbamate of the reductive pathway is not known Fink et al [29] were unable to detect 5 methylbarbituric acid which is a breakdown product of thymine in microorganisms

### *Relation to Valine*

Coon and his associates have demonstrated the production of BAIB from valine [30–34] The reactions are indicated in Fig 24 2 Of particular interest is the BAIB glutamic transaminase reaction studied by Kupiecki and Coon [34] This conversion of BAIB to methylmalonate semialdehyde provides a means of further catabolism of BAIB regardless of its origin

mental evidence to support this concept This mechanism can now be excluded Crumpler et al [2] reported that the blood levels of BAIB in genetic high excretors were no higher than in the rest of the population and considered this as evidence for a defect in tubular reab orption The levels which they found were not reported and it is not clear that they actually detected BAIB in the plasma of any of their subjects The amount of BAIB circulating in the plasma is far less than other amino acids and paper chromatographic methods which can be used for other amino acids in plasma are inadequate for BAIB either in high or low excretors Evered [35] was unable to detect BAIB in the plasma by ion exchange chromatography He computed that the plasma clearance in a high excretor must be at least 85 ml per min indicating little if any tubular reabsorption in these persons Gartler [36] increased the blood levels of BAIB in nonexcretors artificially by oral administration of thymine and found that the plasma clearance actually exceeded the glomerular filtration rate Thus it seems likely that BAIB must be classi fied with tho e substances which are not reabsorbed at all thereby ruling out a renal mechanism for high excretion both in genetic and nongenetic high excretors

*Rate of Formation of BAIB*

In con idering the possibility that increased BAIB excretion results from increased formation from precur ors it is necessary to decide which of the two pos ible pathways—from thymine and from valine—is quanti tatively more important The evidence is meager but favors the thymine pathway Awapara and Shullenberger [9] found that admini tration of 1 gm thymine to a variety of patients suffering from neoplastic disea es results in a manyfold increase in BAIB excretion Similarly Gartler [37] found that ingestion of thymidine thymine dihydrothymine or $\beta$ ureido-isobutyric acid by normal high and low excretors cau es increased excre-tion of BAIB On the other hand the oral admini tration of 500 mg valine to a genetic high excretor and to a low excretor did not result in an in crea e of BAIB in the urine [37] This amount of valine is much less than the amount of other amino acids u ed in tolerance loads and po ibly is inadequate to test the ability of valine to serve as a precur or of BAIB Awapara and Shullenberger [9] al o found an increa e in BAIB excretion in many of their patients after admini tration of nitrogen mustard They interpret this as a con equence of the release of thymine from ti ue and sugge t that excretion of BAIB may be a u eful index of the extent of tissue de truction

This la t ol ervation provides an explanation for the association of high BAIB excretion with certain disease involving increased tissue destruction (e g leukemia march hemoglobinuria) It was early reported that the rate of excretion varied with the clinical state of the patient in

Unfortunately, this has been largely ignored BAIB from the urine of genetic excretors is levorotatory [2] but this in itself does not indicate which pathway is responsible for the production of excessive amounts of BAIB Complicating this picture is the observation by Fink, Henderson and Fink [21] that only 15 per cent of a racemic mixture of BAIB injected intraperitoneally into rats is recovered in the urine This would suggest that both forms can be metabolized Since the BAIB glutamic transaminase appears to be insensitive to the methyl group reacting either with β alanine or with α methyl β alanine (BAIB) it is quite possible that it is also insensitive to the steric configuration of the α-carbon In the case of valine catabolism and undoubtedly in the case of thymine catabolism only one isomer would be formed in vivo

*Relation to Other Amino Acids*

Although BAIB conforms chemically to the definition of an amino acid it should not be grouped metabolically with the other amino acids It has never been isolated from a protein nor is it known to participate in peptide bond formation As will be discussed later it probably does not compete with other amino acids in transport reactions The amino nitrogen does enter the nitrogen pool of the amino acids by means of the transaminase reaction

## CAUSES OF EXCESS BAIB EXCRETION

The nature of the metabolic defect which results in BAIB excretion has not been established either in genetic excretors or in persons suffering from various diseases Indeed there is no a priori reason for supposing that the cause is the same in the two cases On the basis of the known metabolic reactions of BAIB it is possible to suggest several mechanisms by which BAIB excretion might be increased It should be kept in mind that there is virtually no information concerning the quantitative importance of the pathways which have been established nor for that matter is it often known which direction predominates in these frequently reversible reactions

An increased excretion of a metabolite has one of three causes (1) increased formation of that metabolite from its precursors (usually because some alternate pathway of the precursor is blocked), (2) decreased capacity to metabolize the product with the result that the blood levels increase and the substance appears in the urine (3) decreased ability to reabsorb the metabolite from the glomerular filtrate

*Role of Renal Mechanisms*

A renal mechanism has frequently been postulated to account for β aminoisobutyricaciduria but there has never actually been experi

In the absence of additional experimental evidence it is obviously impossible to decide which of the two pathways from thymine or valine is quantitatively important in the excretion of BAIB. The significant evidence will most probably come from tracer studies rather than from loading experiments in which unphysiologic levels of metabolites are achieved. A point which must also be considered is that in conditions of excessive tissue destruction BAIB excretion occurs in persons who are not genetic excretors. This implies that the ability to metabolize BAIB is limited in such persons also, but at a level which permits them to handle normal turnover without appreciable excretion of BAIB. Such a delicate balance is unusual but certainly not impossible.

## GENETICS OF BAIB EXCRETION

### *Detection Methods and the Problem of Classification*

It is impossible to discuss the genetics of BAIB excretion without referring to methods of assay. BAIB was discovered by means of paper chromatography, which has remained the principal means of detection. Ion-exchange methods, as applied by Evered [35], are the most accurate available at present, but genetic studies depend upon the assay of large numbers of samples, and the ion-exchange methods are very time consuming. Of necessity the method of choice has been paper chromatography, in spite of the relatively large error inherent in its use as a quantitative tool. Furthermore, there are several ninhydrin reacting substances which occasionally appear in small quantities in positions on the chromatogram very close to BAIB, and it is not always possible on a single chromatogram to distinguish these substances. For these reasons the trace amounts of BAIB frequently reported are apt to reflect substances other than BAIB.

The difficulties imposed by analytic error are apparent when one attempts to assign each individual to a particular category of excretion. In the previous discussions the terms high and low excretor have been used as if the distribution of excretion rates were discontinuous. It would indeed be convenient if individuals could be assigned to nonoverlapping categories, since conventional Mendelian analysis is based on attributes rather than on continuous variables. The evidence on this point is contradictory. Some find evidence of bimodality [46], while others find no evidence for bimodality [43]. In populations where the frequency of high excretors is low, a very large population sample would be required in order to demonstrate a significant separation between the two groups. However, even in populations where the frequency of high excretors is high, the evidence for a clear separation between high and low excretors is not convincing. Cartler [37], using a paper electrophoretic technique for estimating BAIB, has obtained results which he considers to be

contrast with the relatively constant rate of excretion of normal high excretors [3] High BAIB excretion is frequently observed in hospitalized patients particularly in small children suffering from obscure metabolic disorders [38] In the absence of other family information it is probably best to consider these patients as having excessive tissue destruction or at least altered DNA metabolism

*Metabolic Disposal of BAIB*

There is certainly no reason to suppose that genetic high excretors have increased tissue destruction It is conceivable that their DNA metabolism may differ from that of low excretors but Gartler [36, 37] has provided evidence that the difference is in the handling of metabolites subsequent to the reduction of thymine This is most clearly indicated by the results of oral and intravenous administration of DL-BAIB Low excretors retain most of the BAIB while high excretors dispose of it in the urine Since the conversion of β ureidoisobutyric acid to BAIB is essentially irreversible there is no reason to implicate reactions of the thymine pathway prior to the actual formation of BAIB

It seems necessary to postulate a block in the conversion of BAIB to methylmalonate semialdehyde or in the further metabolism of the latter to propionate etc If the transaminase reaction is blocked then the catabolism of valine should have little influence on the excretion of BAIB On the other hand if the block occurs subsequent to methylmalonate semialdehyde and assuming this pathway to be the principal means of valine breakdown, then valine should also contribute to the BAIB excreted

It is informative to consider the quantities of the various possible precursors which would be required to account for the urinary BAIB in a high excretor If an excretion rate of 200 mg BAIB per day is assumed as a reasonable value for a high excretor then 245 mg thymine 470 mg thymidine 1 94 gm DNA or 227 mg valine would have to be converted with 100 per cent efficiency to urinary BAIB to account for this rate If the DNA is considered to represent tissue breakdown then approximately 400 to 800 gm fresh tissue would have to be destroyed per day to produce this much DNA (assuming concentrations of 25 to 50 μg DNA phosphorus per 100 mg fresh tissue [39]) Such a turnover rate perhaps is not unreasonable [40] It is quite possible that even if thymine is responsible for all the BAIB produced it does not all come from completed DNA Valine is an essential amino acid and arises entirely from the diet The average daily per capita consumption of valine is about 5 gm [41] of which only about 10 per cent is needed to maintain positive nitrogen balance [42] Thus a diversion of about 5 per cent of the valine into BAIB would account for the amount of BAIB excreted even in high excretors

amounts of BAIB behaved as a recessive trait The only mating of high excretor with high excretor produced three high-excretor offspring

A subsequent study of an Italian population by Calchi Novati et al [45] using the same procedures as those of Harris revealed a frequency of 40 high excretors in a population of 711 individuals over 4 years of age Again the analysis of family material indicated consistency with the hypothesis that high excretors are homozygous for a recessive gene The only mating of high excretor with high excretor produced a single high excretor offspring

Gartler [47] studied a series of New York families using 30 mg per gm creatinine as the dividing line There were no matings of high excretor with high excretor but the distribution of offspring types in the other matings was consistent with the inheritance of high excretion as a recessive trait

Grouchy and Sutton [48] studied the inheritance of BAIB excretion in 17 families of Chinese or Japanese extraction This population is particularly useful since the frequency of high excretors is very much higher in it than in Caucasian populations (Fig 24 2) In the absence of evidence for a distinct antimode statistical techniques were applied which are valid for multigenic inheritance These showed a high correlation among siblings and between offspring and parents confirming the essentially genetic character of this trait Although neither the type of segregation within families nor the type of progeny produced by the various matings was entirely compatible with a simple two-allele hypothesis the evidence did not exclude the possibility that a major portion of the genetic variation could be accounted for by a simple two-allele Mendelian system

Gartler Firschein and Kraus [46] reported a series of family studies on a Black Carib population which has a high frequency of BAIB excretors They chose as their dividing line the value

$$\log (\text{O D BAIB}/\text{O D glycine}) = 1\ 40$$

where O D = the optical density of the amino acid spots measured on paper chromatograms

The value 1 40 is the point at which an antimode occurred in this population The results again were in general agreement with the hypothesis that high excretion results from homozygosity for a recessive gene In this instance there were 5 matings of high excretor with high excretor producing 24 high-excretor offspring and 2 low-excretor offspring The latter 2 low-excretor offspring are inconsistent with the simple 2 allele system but the true family relationships may not have been ascertained

In summarizing the foregoing genetic studies perhaps the best conclusion is that the major variation in BAIB excretion is under the control of a very few genes possibly a single pair of alleles and that high excretion results from homozygosity for a recessive allele There still appear to be

superior to those obtained by the chromatographic methods As yet no family studies with this method have been reported

*Environment Sex Diet and Age*

Before proper genetic studies can be undertaken it is necessary to evaluate the environmental factors which may alter the trait under study Experiments have already been described in which the BAIB excretion is altered by ingestion of large amounts of thymine, dihydrothymine or β-ureidoisobutyric acid Such situations are not likely to arise outside a laboratory, however, and all the other evidence favors the view that diet is ordinarily ineffective in changing the level of BAIB excretion On the assumption that thymine is the precursor of BAIB one study was carried out by placing two individuals one a high excretor and the other a low excretor, on a meat free diet for 1 week [38] Although certain other features of the excretion pattern changed the BAIB excretion remained constant and quite different for each individual In another study of the excretion of BAIB within families, no correlation was found between husbands and wives but there was a significant correlation between parents and offspring [43] This is good evidence that BAIB excretion is not readily influenced by usual dietary variations It seems best to consider that excretion of BAIB is the result of endogenous processes not readily influenced by either quantity or quality of food Other environmental factors have not been investigated for their influence on BAIB excretion but in view of the individual consistency in excretion rates there is little reason to suppose that such factors play a significant role

Many metabolites vary considerably with age or sex but in the case of BAIB these too appear to be unimportant None of the studies in which sex differences were investigated has reported a significant difference [4 43–47] For the most part no age differences have been reported [4 43 46] although several investigators have found increased levels of BAIB among young children (less than 4 or 5 years of age) [10 44 45 47] The importance of this increase in young children is not known but it suggests caution in the interpretation genetic or otherwise of the excretion levels of young children

*Genetic Control*

The first study of the genetic control of BAIB excretion was that of Harris [4] in England He selected as his dividing line between high and low excretors the amount of BAIB which reacted on paper with ninhydrin to give a spot equal in intensity to the alanine spot of the same urine sample This amount was estimated to be about 70 mg per gm creatinine Of the 345 persons examined 33 (9 6 per cent) were high excretors by this criterion In the family studies the excretion of large

amounts of BAIB behaved as a recessive trait. The only mating of high excretor with high excretor produced three high-excretor offspring.

A subsequent study of an Italian population by Calchi Novati et al. [45] using the same procedures as those of Harris revealed a frequency of 40 high excretors in a population of 711 individuals over 4 years of age. Again the analysis of family material indicated consistency with the hypothesis that high excretors are homozygous for a recessive gene. The only mating of high excretor with high excretor produced a single high excretor offspring.

Gartler [47] studied a series of New York families using 30 mg per gm creatinine as the dividing line. There were no matings of high excretor with high excretor, but the distribution of offspring types in the other matings was consistent with the inheritance of high excretion as a recessive trait.

Grouchy and Sutton [43] studied the inheritance of BAIB excretion in 17 families of Chinese or Japanese extraction. This population is particularly useful since the frequency of high excretors is very much higher in it than in Caucasian populations (Fig. 24-2). In the absence of evidence for a distinct antimode, statistical techniques were applied which are valid for multigenic inheritance. These showed a high correlation among siblings and between offspring and parents, confirming the essentially genetic character of this trait. Although neither the type of segregation within families nor the type of progeny produced by the various matings was entirely compatible with a simple two-allele hypothesis, the evidence did not exclude the possibility that a major portion of the genetic variation could be accounted for by a simple two-allele Mendelian system.

Gartler, Firschein, and Kraus [46] reported a series of family studies on a Black Carib population which has a high frequency of BAIB excretors. They chose as their dividing line the value

$$\log (\text{O.D. BAIB}/\text{O.D. glycine}) = 1.40$$

where O.D. = the optical density of the amino acid spots measured on paper chromatograms.

The value 1.40 is the point at which an antimode occurred in this population. The results again were in general agreement with the hypothesis that high excretion results from homozygosity for a recessive gene. In this instance there were 5 matings of high excretor with high excretor producing 21 high-excretor offspring and 2 low-excretor offspring. The latter 2 low-excretor offspring are inconsistent with the simple 2-allele system, but the true family relationships may not have been ascertained.

In summarizing the foregoing genetic studies, perhaps the best conclusion is that the major variation in BAIB excretion is under the control of a very few genes, possibly a single pair of alleles, and that high excretion results from homozygosity for a recessive allele. There still appear to be

other factors either genetic or nongenetic or both, which can influence the amount of BAIB excreted As new assay techniques become available and the experimental error can be reduced the nature of the genetic factors should also become much clearer

## RACIAL VARIATION IN BAIB EXCRETION

As with many other inherited traits the frequency of the genes controlling BAIB excretion varies considerably in different populations This

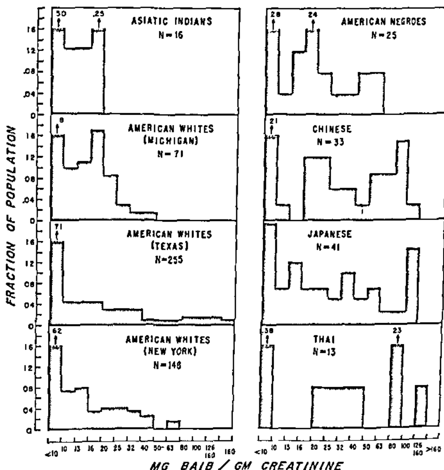



Fig 24 3 Distribution of excretion rates of BAIB in various populations References are given in Table 24 1

was recognized first during the comparison of the amino acid excretion patterns of a small group of Chinese with those of Americans of European extraction [48] Instead of the rare high excretor found among Europeans the Chinese were found to have a very high frequency of high excretors

This difference among populations has been confirmed and extended and the results of some of the studies are summarized in Fig 24 3 Another means of expressing these differences is in the proportion of individuals whose level of excretion is greater than some value In Table 24 1 are shown the results expressed in this manner with studies of some additional populations whose published values were not expressed in a way which permitted their inclusion in Fig 24 3 It is apparent that the highest excretors occur among the Orientals and among the American Indians with intermediate values for Negroes Allison Blumberg and

TABLE 24-1 FREQUENCIES OF INDIVIDUALS EXCRETING BAIB AT LEVELS GREATER THAN 30 MG PER GM CREATININE IN VARIOUS POPULATIONS

| *Population* | *Frequency of excretors* | *No of persons* | *Reference* |
|---|---|---|---|
| American white | | | |
| Michigan | 0.03 | 71 | [38] |
| Texas | 0.078 | [illegible] | [illegible] |
| New York | 0.115 | 148 | [4 ] |
| Asiatic Indians | 0.00 | 16 | [38] |
| Negroes | | | |
| Michigan | 0.20 | 25 | [38] |
| Black Caribs | 0.316 | 285 | [*10*] |
| New York | 0.15 | 38 | [*10*] |
| Chinese | 0.45 | 33 | [38] |
| Japanese | 0.415 | 41 | [38] |
| Thai | 0.46 | 13 | [38] |
| Apaches (Arizona) | 0.416 | 113 | [*10*] |

Gartler [*49*] have also reported high values for the Athabascan speaking Indians of Alaska but found that the Alaskan Eskimos are intermediate between the Indians and whites

The existence of such genetic polymorphism among all the populations studied can be interpreted as indicating a very early origin of the (two) alleles with the heterozygous individuals possibly enjoying some selective advantage over their homozygous neighbors The possibility of independent mutations at this locus cannot be excluded of course The nature of the selective factors is unknown

## SUMMARY

1 β-Aminoisobutyric acid (BAIB) occurs in the urine of some persons in amounts up to 200 to 300 mg per day either as an inherited trait or as a consequence of some disease process

2 Enzymes are present in animal tissues which form BAIB from thymine and from valine but present evidence is not conclusive as to the

actual origin of urinary BAIB In some disease states the increased BAIB excretion probably reflects excessive tissue destruction with concomitant high DNA catabolism

3 BAIB is not reabsorbed by the renal tubules either in low or high excretors of BAIB The failure of genetic low excretors to excrete ingested BAIB while high excretors do excrete ingested BAIB is evidence that a block exists in the further metabolism of BAIB This may be either in the transamination to methylmalonate semialdehyde or in subsequent steps

4 The evidence for dimorphism in the excretion rates of BAIB is not clear cut although recently developed techniques may resolve some of the earlier reported discrepancies Nevertheless the principal genetic variation seems to be largely explained on the basis of a two-allele system with high excretors being homozygous for a recessive gene There still remains variation either genetic or nongenetic which is not explained by such a system

5 Sex and diet do not appear to influence the excretion of BAIB Very young children are reported to be high excretors more often than adults but age does not appear to affect the excretion rate among persons over 5 years of age The frequencies of persons who excrete large amounts of BAIB vary with the population under study The lowest frequencies are found in white populations and the highest in Mongolians and American Indians with Negroes intermediate

## BIBLIOGRAPHY


1 Dent C E A study of the behavior of some sixty amino-acids and other ninhydrin reacting substances on phenol collidine' filter paper chromatograms with notes as to the occurrence of some of them in biological fluids Biochem J 43 168 1948

2 Crumpler H R Dent C E Harris H and Westall R G β Aminoisobutyric acid (α-methyl β alanine) a new amino-acid obtained from human urine Nature 167 307 1951

3 Fink K Henderson R B and Fink R M Beta aminoisobutyric acid a possible factor in pyrimidine metabolism Proc Soc Exper Biol & Med 78 135 1951

4 Harris H Family studies on the urinary excretion of β aminoisobutyric acid Ann Eugenics 18 43 1953

5 Fink K A substance occasionally found as a major ninhydrin reacting component of urine Proc Soc Exper Biol & Med 76 692 1951

6 Ishihara I and Komori Y An unidentified amino acid excreted in large amounts in the urine of leukemic patients Igaku to Seibutsugaku 22 1 1952

7 Awapara J and Sato Y Paper chromatography of urinary amino acids Clin chim acta 1 75 1956

8 Awapara J Urinary amino acid excretion in leukemia in *The Leukemias Etiology Pathophysiology and Treatment* edited by J W Rebuck F H Bethell and R W Monto pp 303–310 Academic Press Inc New York 1957

9 Awapara J and Shullenberger C C Urinary excretion of β-aminoisobutyric acid after administration of thymine and nitrogen mustard Clin chim acta 2 199 1957

10 Gartler S M Firschein I L and Gida pow T Some genetical and anthropological considerations of urinary β aminoisobutyric acid excretion Acta genet 6 435 1957

11 Ishihara I Komori Y and Yokoo Y Urinary excretion of β-aminoisobutyric acid and generaliz l aminoa i luria in pulmonary tuber ulosi Igaku to Seibutsugaku 29 50 1953
1 Harr C M B and Sandler M Amino-aciduria in march haemoglobinuria Lancet 266 70 1954
13 Dent C L and Walshe J M Amin acid metabolism in liver disease In Liver Disease (Ciba Foundation Symposium) edited by S Sherlock and G E W Wolstenholme pp -30 McGraw Hill—Blakiston New York 1951
14 Wilson V K Thomson M L and Dent C E Amino aciduria in lead poisoning A case in childhood Lancet 265 66 1953
15 Cain L Exploration of metabolic patterns in mentally deficient children Univ Texas Bull No 5109 198 1951
16 Wright S W and Fink K The excretion of β-aminoisobutyric acid in normal mongoloid and non mongoloid mentally defective children Am J Ment Deficiency 61 530 1957
17 Grouchy J and Sutton H E Unpublished results
18 Fink K and Fink R M Some factors influencing excretion of beta aminoisobutyric acid (BAIB) Fed Proc 17 219 1958
19 Rubini J R Cronkite E P Bond V P and Fliedner T M Urinary e cretion of beta aminoisobutyric acid (BAIBA) in irradiated human beings Proc Soc Exper Biol & Med 100 130 1959
19a Berry H K Excretion of β aminoisobutyric acid (B-AIB) Metabolism 9 373 1960
20 Tallan H H Moore S and Stein W H Studies on the free amino acids and related compounds in the tissues of the cat J Biol Chem 211 927 1954
21 Fink K Henderson R B and Fink R M β-Aminoisobutyric acid in rat urine following administration of pyrimidines J Biol Chem 197 441 1952
22 Fink R M Fink K and Henderson R B β Amino acid formation by tissue slices incubated with pyrimidines J Biol Chem 201 349 1953
23 Fink R M McGaughey C Cline R E and Fink K Metabolism of intermediate pyrimidine reduction products in vitro J Biol Chem 218 1 1956
24 Fink K Excretion of pyrimidine reduction products by the rat J Biol Chem 218 9 1956
25 Wallach D P and Grisolia S The purification and properties of hydropyrimidine hydrase J Biol Chem 226 277 1957
26 Canellakis E S Pyrimidine metabolism I Enzymatic pathways of uracil and thymine degradation J Biol Chem 221 315 1956
27 Grisolia S and Wallach D P Enzymic interconversion of hydrouracil and β-ureidopropionic acid Biochim et biophys acta 18 449 1955
28 Caravaca J and Grisolia S Enzymatic decarbamylation of carbamyl β-alanine and carbamyl β-aminoisobutyric acid J Biol Chem 231 357 1958
29 Fink K Cline R E Henderson R B and Fink R M Metabolism of thymine (methyl C or 2-C ) by rat liver in vitro J Biol Chem 221 4 5 1956
30 Coon M J Enzymatic synthesis of branched chain acids from amino acids Fed Proc 14 6 1955
31 Robinson W G Nagle R Bachhawat B K Kupiecki F P and Coon M J Coenzyme A thiol esters of isobutyric methacrylic and β-hydroxyisobutyric acids as intermediates in the enzymatic degradation of valine J Biol Chem 224 1 1957
32 Robinson W G and Coon M J The purification and properties of β hydroxyisobutyric dehydrogenase J Biol Chem 225 511 1957
33 Rendina G and Coon M J Enzymatic hydrolysis of the coenzyme A thiol esters of β hydroxypropionic and β-hydroxyisobutyric acids J Biol Chem 225 5 3 195
34 Kupiecki F P and Coon M J The enzymatic synthesis of β-aminoisobutyrate a product of valine metabolism and of β-alanine a product of β-hydroxypropionate metabolism J Biol Chem 229 43 1957
35 Evered D F The excretion of amino acids by the human A quantitative study with ion-exchange chromatography Biochem J., 62 416 1956

36 Gartler S M An investigation into the biochemical genetics of β-aminoisobutyric aciduria Am J Human Genetics 11 257 1959
37 Gartler S M A metabolic investigation of urinary β-aminoisobutyric acid excretion in man Arch Biochem 80 400 1959
38 Sutton H E Unpublished results
39 Leslie I The nucleic acid content of tissues and cells in *The Nucleic Acids* edited by E Chargaff and J N Davidson vol II pp 1 10 Academic Press Inc New York 1955
40 *Leblond C P and Walker B E Renewal of cell populations Physiol Revs* 36 255 1956
41 Block R J and Weiss K W *Amino Acid Handbook* p 175 Charles C Thomas Springfield Ill 1956
42 Rose W C Wixom R L Lockhart H B and Lambert G F The amino acid requirements of man XV The valine requirement summary and final observations J Biol Chem 217 987 1955
43 Grouchy J and Sutton H E A genetic study of β-aminoisobutyric acid excretion Am J Human Genet 9 76 1957
44 Berry H K Variations in urinary excretion patterns in a Texas population Am J Phys Anthropol 11 [illegible] 1953
45 Calchi Novati C Ceppellini R Biancho I Silvestroni E and Harris H β-Aminoisobutyric acid excretion in urine A family study in an Italian population Ann Eugenics 18 335 1954
46 Gartler S M Firschein I L and Kraus B S An investigation into the genetics and racial variation of BAIB excretion Am J Human Genet 9 200 1957
47 Gartler S M A family study of urinary β-aminoisobutyric acid excretion Am J Human Genet 8 120 1956
48 Sutton H E and Clark P J A biochemical study of Chinese and Caucasoid Am J Phys Anthropol 13 53 1955
49 Allison A C Blumberg B S and Gartler S M Urinary excretion of β-aminoisobutyric acid in Eskimo and Indian populations of Alaska Nature 183 118 1959

Part Seven

# Diseases Primarily Manifest as Abnormalities in Metal Metabolism

# Chapter 25

## Wilson's Disease*

*Alexander G. Bearn*

It is quite certain that if this book had been conceived 25 years ago it would have been comfortably slimmer and would not have included a chapter on Wilson's disease. The view would have been almost unanimously held that the disease could not be considered either inherited or metabolic. Although during the past 25 years the nature of the disease has become greatly clarified, the basic metabolic defect underlying its cause remains obscure.

Wilson's disease (hepatolenticular degeneration) is a relatively rare disease occurring predominantly in young people. It is characterized by cirrhosis of the liver and degenerative changes in the brain, particularly in the basal ganglia. The greenish brown Kayser-Fleischer rings seen in the periphery of the cornea are pathognomonic of Wilson's disease. Genetic studies have revealed that the condition is inherited in an autosomal recessive fashion.

### CLINICAL FEATURES

In 1912 Kinnier Wilson published his monograph entitled "Progressive Lenticular Degeneration: A Familial Nervous Disease Associated with Cirrhosis of the Liver" [1]. While still a young man Wilson had under his care a patient who died after a long illness characterized by muscular rigidity, tremor, and forced grimacing. Cirrhosis of the liver and gross degenerative changes in the lenticular nucleus of the brain were found at autopsy. On searching the literature, Wilson found other cases which were clinically similar. Six years earlier Gowers, in a paper entitled "Tetanoid Chorea Associated with Cirrhosis of the Liver," described

* The author is indebted to Professor L. S. Penrose for his helpful discussions concerning the interpretation of the genetic data and to Professor Sheila Sherlock and Dr. James L. German III for their assistance in the preparation of the manuscript.

two patients whose symptoms were similar to those of Wilson's patient [2]. A review of the literature at that time disclosed that the disease had been seen sporadically in the past but had escaped systematic study. The first case of recognized Wilson's disease was probably described by Frerichs in his classic monograph on liver disease in 1861 [3]. The patient, a young boy, suffered from severe liver disease associated with violent tremors and convulsions and died at the age of 10. An autopsy revealed cirrhosis of the liver. The age of the patient and the nature of his symptoms make the diagnosis of Wilson's disease, although unproved, highly likely.

The clinical characteristics of Wilson's disease may be so unique that in many instances the diagnosis is not in doubt. Unfortunately, the "typical case" is becoming less frequent as understanding of the varied clinical manifestations of the disease increases.

*Lenticular Degeneration*

This form of the disease is the one seen by Wilson and overemphasized by subsequent observers. In fact, classic lenticular degeneration is a severe but relatively uncommon form of the disease which occurs predominantly in young adults. Spasticity, rigidity, dysarthria, and dysphagia are the common presenting symptoms. Tremor is usually less pronounced, and clinical evidence of hepatic disease is minimal or absent, although, as in all varieties of Wilson's disease, pathologic evidence of cirrhosis is almost invariable. Acute unexplained febrile episodes were recognized by Wilson in some of his patients and may usher in the terminal phase of the disease.

*Pseudosclerosis*

Unnecessary and frequently acrimonious nosologic debate obscured the existence of this variety of hepatolenticular degeneration during the early part of this century. It is now clear that what has been known as pseudosclerosis of Westphal [4] is a variant of Wilson's disease in which tremor is usually the major disabling symptom, while rigidity and spasticity are less marked. Symptoms referable to hepatic dysfunction are uncommon, but pathologic evidence of cirrhosis can invariably be found, and impairment of Bromsulfalein excretion can frequently be demonstrated [5]. Other biochemical evidence of disturbed liver function is usually slight.

*Kayser Fleischer Ring*

Despite the brilliance of Wilson's clinical acumen, the only pathognomonic sign of the disease escaped him. Ten years before the publication of Wilson's paper Kayser had observed a greenish yellow ring at the limbus of the cornea in a patient diagnosed as having multiple sclerosis. A year later Fleischer reported a corneal ring as an integral part of a

neurologic disease associated with cirrhosis of the liver In a series of papers [6-8] Fleischer proposed the concept that the changes in the eye brain and liver were all due to a common metabolic cause The Kayser Fleischer ring is still rightly regarded as the single most important diagnostic sign of the disease The ring is not always complete and is usually most marked at the superior and inferior aspects of the cornea Although with experience most Kayser Fleischer rings can be seen by inspection of the cornea its presence can be definitely excluded only by a slit lamp examination In a very few patients careful examination of the eye will reveal a *Sonnenblumenkatarakt* Similar cataracts are known to follow the intraocular localization of a foreign body containing copper

*Cirrhosis of the Liver*

The hepatic form of Wilson's disease in which there is evidence of liver abnormality with minor or absent neurologic signs has been recognized as a clinical variant for many years [9] Its frequency and significance have recently become increasingly appreciated [10] In a series of 32 cases collected at the Rockefeller Institute between 1951 and 1958 8 (25 per cent) could be classified as belonging to this group It seems likely that a significant but unknown proportion of patients with juvenile cirrhosis actually suffer from Wilson's disease No patient with juvenile cirrhosis has been satisfactorily examined unless the presence of Kayser Fleischer rings has been diligently sought and the biochemical tests necessary for the diagnosis of Wilson's disease have been performed The cirrhosis is similar pathologically to that seen in the pseudosclerotic variety of the disease but is generally more severe Signs of portal hypertension can frequently be demonstrated In some patients with Wilson's disease the presenting symptom is massive hemorrhage due to rupture of esophageal varices Hepatic coma may usher in the terminal phase Ascites rarely occurs except in the later stages of hepatic decompensation

*Other Clinical Variants*

A sizable proportion of patients with Wilson's disease may present with symptoms suggesting schizophrenia others may develop less pronounced abnormalities in behavior or personality Tragic ignorance of this form of the disease has resulted in needless shock therapy and unnecessary detention in psychiatric hospitals

Occasionally patients may develop epileptic seizures usually of the Jacksonian type Hemiplegia is not so rare as is commonly supposed A curious comatose state may persist for several weeks and does not necessarily herald an immediate fatal outcome

*Etiology*

Two hypotheses are current concerning the probable pathogenetic mechanism of Wilson's disease The first and presently more favored

hypothesis regards the disease as a primary disturbance in copper metabolism The second ascribes the disease to a more generalized disorder of tissue protein metabolism Neither of these two hypotheses is entirely satisfactory and both philosophy and common sense suggest that the final view will incorporate features of both For purposes of clarity in exposition these two hypotheses will be dealt with separately and the defects of each emphasized

## NORMAL METABOLISM OF COPPER

Copper is an ubiquitous element Under normal circumstances the average individual consumes between 2 5 and 5 0 mg copper per day Foods with high copper content include liver shellfish, nuts and chocolate Cow's milk usually contains only a small amount of copper and has been used successfully to induce copper deficiency in certain animals In most foodstuffs copper is bound principally to proteins and amino acids Copper may be present in significant quantities in drinking water particularly if the pipes are copper lined

### *Ceruloplasmin Copper*

Copper in the serum is bound to the plasma proteins Approximately 98 per cent of the circulating serum copper in man is bound to an $\alpha_2$-globulin Purification of the $\alpha_2$-globulin fraction has resulted in the isolation of a copper protein which because of its blue color is called ceruloplasmin

Ceruloplasmin has a molecular weight of about 151 000 and contains eight copper atoms per molecule [*11 12*] Holmberg and Laurell [*13, 14*] have described some of its properties The protein is an oxidase with many properties in common with plant laccases Among the variety of compounds toward which it exhibits oxidase activity are biologically important substances such as epinephrine and serotonin as well as synthetic substrates [*15*] The greatest oxidase activity has been demonstrated using as substrate the dye paraphenylenediamine The enzymatic activity may be accelerated by low concentrations of a variety of monovalent anions including chloride bromide and acetate higher concentrations of these may inhibit Some polyvalent ions phosphate and citrate, for example may inhibit the enzymatic reaction and cause a prolonged induction period Whether ceruloplasmin exhibits enzyme function in vivo is uncertain

Procedures designed to dissociate copper from ceruloplasmin have resulted in loss of enzymatic activity It has been demonstrated by the use of $Cu^{64}$ that under appropriate conditions half of the eight copper atoms of each ceruloplasmin molecule can exchange with ionic copper [*16*] This will take place only if the protein first is reduced to its colorless form Ceruloplasmin in vivo may under certain circumstances exchange

its copper with copper absorbed from the gastrointe tinal tract [17] If this mechanism can be demonstrated to occur under phy iologic conditions ceruloplasmin would perform a role in copper tran port similar to that of transferrin in iron transport It is al o possible that ceruloplasmin is a storage protein Since a gross deficit of cerulopl smin has been demonstrated in a healthy carrier of the abnormal gene of hepatolenticular degeneration it is unlikely that its enzymatic function is vital to the organism The relationship of ceruloplasmin to mental disease [18] remains too speculative for further comment It is clear that the relationship, if any, is both tenuous and indirect [19]

**Ceruloplasmin Determination** Serum ceruloplasmin can be measured in a variety of ways Holmberg and Laurell originally demonstrated a positive correlation between the copper content of the serum and its oxidase activity using paraphenylenediamine as a substrate The oxidase activity can be measured directly in a Warburg apparatus and recorded in terms of micromoles of $O_2$ uptake per milliliter per hour Alternatively the oxidase activity can be measured colorimetrically Ravin has out lined a rapid method for ceruloplasmin determination using this prin ciple [20] Other methods for estimating this protein depend upon the blue color Measurement of the optical density before and after destruc tion of the blue color by ascorbic acid or cyanide is an index of the con centration of ceruloplasmin [21] Ceruloplasmin can also be measured immunologically [22] Thi of course is dependent upon the availability of a pure preparation of ceruloplasmin which can be used as an antigen In 1958 Broman [23] reported the existence of multiple ceruloplasmins in normal plasma with similar enzymatic properties The main com ponent accounts for approximately 80 per cent of the total cerulopla min The heterogeneity of ceruloplasmin ob erved by Broman and recently confirmed by Sass Kortsak [23a] may have important implication in the understanding of the mech anism of gene action in Wilson s di ease

*Nonceruloplasmin Copper*

It can be shown by salt fractionation and more precisely by electro phoretic techniques that the nonceruloplasmin copper is bound to serum albumin [24] The physical and chemical characteristics of the copper albumin complex have been exten ively studied [25] In general it may be stated that the main copper binding sites of the albumin molecule are the imidazole groups but some weaker binding also occurs at the carboxyl group The extent of the binding depends on the pH and the competitive effect of other anions in solution Heating to approximately 100 C does not di turb the ability of protein to form complexes with copper in contrast to its effect on protein anion complexes The essential require ments for binding appear to be the presence of appropriate residues and favorable electro tatic conditions of the molecule

hypothesis regards the disease as a primary disturbance in copper metabolism The second ascribes the disease to a more generalized disorder of tissue protein metabolism Neither of these two hypotheses is entirely satisfactory, and both philosophy and common sense suggest that the final view will incorporate features of both For purposes of clarity in exposition these two hypotheses will be dealt with separately and the defects of each emphasized

## NORMAL METABOLISM OF COPPER

Copper is an ubiquitous element Under normal circumstances the average individual consumes between 2 5 and 5 0 mg copper per day Foods with high copper content include liver shellfish, nuts and chocolate Cow s milk usually contains only a small amount of copper and has been used successfully to induce copper deficiency in certain animals In most foodstuffs copper is bound principally to proteins and amino acids Copper may be present in significant quantities in drinking water, particularly if the pipes are copper lined

### *Ceruloplasmin Copper*

Copper in the serum is bound to the plasma proteins Approximately 98 per cent of the circulating serum copper in man is bound to an $\alpha_2$-globulin Purification of the $\alpha_2$-globulin fraction has resulted in the isolation of a copper protein which because of its blue color is called ceruloplasmin

Ceruloplasmin has a molecular weight of about 151 000 and contains eight copper atoms per molecule [11 12] Holmberg and Laurell [13 14] have described some of its properties The protein is an oxidase with many properties in common with plant laccases Among the variety of compounds toward which it exhibits oxidase activity are biologically important substances such as epinephrine and serotonin as well as synthetic substrates [15] The greatest oxidase activity has been demonstrated using as substrate the dye paraphenylenediamine The enzymatic activity may be accelerated by low concentrations of a variety of monovalent anions including chloride bromide and acetate higher concentrations of these may inhibit Some polyvalent ions phosphate and citrate for example may inhibit the enzymatic reaction and cause a prolonged induction period Whether ceruloplasmin exhibits enzyme function in vivo is uncertain

Procedures designed to dissociate copper from ceruloplasmin have resulted in loss of enzymatic activity It has been demonstrated by the use of $Cu^{64}$ that under appropriate conditions half of the eight copper atoms of each ceruloplasmin molecule can exchange with ionic copper [16] This will take place only if the protein first is reduced to its colorless form Ceruloplasmin in vivo may under certain circumstances exchange

copper [30] The liver, central nervous system and kidney have the highest copper content (Table 25.2) The muscles and bones because of their total mass contain over 50 per cent of the total body copper Curiously, the fetal liver contains about ten times the amount of copper found in an adult liver The copper content of the liver of a child reaches the level found in a young adult by the age of 10

Several copper proteins have been isolated from the liver Mann and Keilin isolated a colorless copper protein from the liver of the ox [31]

TABLE 25-2 MEAN COPPER CONTENT OF VARIOUS ORGANS IN NORMAL SUBJECTS AND PATIENTS WITH WILSON'S DISEASE

| *Organ* | *Normal subjects* | *Patients with Wilson's disease* |
|---|---|---|
| Liver | 0.5 | 10.0 (5.0–[illegible]0.0) |
| Kidney | 0.3 | 1.0 (0.8–8.0) |
| Basal ganglia | 0.6 | 3.0 (2.0–8.0) |
| Cortex | | |
| White | 0.5 | 5.0 (3.0–6.0) |
| Gray | 0.4 | 6.0 (4.0–8.0) |

All values expressed as mg per 100 gm wet weight

This protein was not crystallized and accounted for only a part of the total copper present in the liver Mohamed and Greenberg isolated an apparently crystalline copper protein from horse liver The protein was blue contained 0.30 to 0.40 per cent copper and had an estimated molecular weight of 30 000 to 40 000 This protein has been named hepatocuprein unlike ceruloplasmin it does not exhibit any oxidase activity [32]

The nature of the copper proteins of normal brain (cerebrocuprein) has been extensively studied by Huntington Porter [33-35] Thus far three copper-containing fractions of normal brain have been isolated and their properties described Fraction I is extracted with acetate buffer at pH 4.5 fraction II is obtained by extraction of the residue from fraction I with water at pH 3.5 at very low ionic strength the final residue fraction is designated fraction III From fraction I has been isolated a green copper-containing protein named cerebrocuprein I This protein contains 0.25 to 0.30 per cent copper and appears 85 per cent homogeneous by ultracentrifugal and electrophoretic techniques It has a molecular weight of approximately 30 000 to 40 000 Cerebrocuprein I exhibits two rather singular properties Copper is not released from cerebrocuprein I by dialysis at pH 3.0 and approximately 15 per cent of its copper reacts directly with diethyldithiocarbamate It is not yet known with certainty whether this direct reacting moiety of normal cerebrocuprein I is of physiologic importance or whether it is an artifact Copper is widely di

Ionic copper reacts with diethyldithiocarbamate to form a characteristic yellow color Copper in ceruloplasmin will not react with this reagent unless the copper is first released from the ceruloplasmin The copper which is bound to albumin reacts directly with this reagent and permits the detection and quantitation of the albumin bound copper This fraction has been designated the "direct reacting copper by Cartwright and his associates [26] It seems probable that although the total amount of albumin copper does not normally greatly exceed 5 per cent of the total serum copper it performs the essential task of transporting copper from the intestinal tract to the various tissues of the body and is metabolically the most active fraction of the serum copper

### *Erythrocuprein*

Copper is a normal component of adult red cells where its function is unknown At least 80 per cent of the copper in erythrocytes is present

TABLE 25-1 A COMPARISON OF THE PHYSICAL AND CHEMICAL PROPERTIES OF ERYTHROCUPREIN AND CERULOPLASMIN

| *Property* | *Erythrocuprein* | *Ceruloplasmin* |
|---|---|---|
| Color | Nearly colorless | Blue |
| Absorption maxima | 655 265 mμ | 605 280 mμ |
| Molecular weight | 33 000 | 151 000 |
| Percentage of copper | 0 32–0 36 | 0 32–0 34 |
| Atoms of copper per molecule | 2 | 8 |
| Isoelectric point | 5 3 | 4 4 |

SOURCE Adapted from G E Cartwright et al See references [27–29]

in the form of erythrocuprein a nearly colorless protein recently isolated from human red cells by Cartwright and his colleagues [27–29] A comparison of some of the physical properties of erythrocuprein and ceruloplasmin is shown in Table 25 1

In copper deficient animals a severe anemia develops which is morphologically similar to the anemia of iron deficiency The erythrocytes of the copper deficient pig have a shortened survival time but when transfused into a normal pig they have approximately the normal life span Erythrocytes transfused from a normal to a copper deficient pig also survive normally Such evidence suggests that the shortened survival time of erythrocytes in copper-deficient animals is not due to an extracorpuscular abnormality Rapid exchange can take place between the serum copper and the red cell copper both in vivo and in vitro [27]

### *Tissue Copper*

It is hardly surprising that copper is widely distributed in all body tissues The body of a normal adult contains approximately 150 mg

1915 when Glazebrook reported a patient with Wilson's disease whose liver and brain at autopsy revealed an increased copper concentration and whose serum copper was considered elevated [41] (In view of subsequent findings the increased serum copper reported may possibly have been in error ) In all events the observations of Glazebrook proved to have catalytic consequences and a bewildering spate of papers on copper metabolism ensued The next major event was the chance finding by Mandelbrote and his coworkers [42] that a patient suffering from Wilson's disease excreted an increased quantity of copper in the urine Numerous observers have now amply confirmed both the increased tissue copper and the high urinary copper excretion Considerably more disagreement has developed in regard to the serum copper concentration in Wilson's disease Although initial observers reported both normal and elevated levels it was subsequently shown that the levels were almost invariably decreased [43] Recently the wheel has turned full circle and several patients with unequivocal Wilson's disease have now been observed in whom the serum copper is unquestionably normal [44 45] whether this is normal ceruloplasmin will be discussed more fully later

*Serum Ceruloplasmin*

Almost simultaneously in different laboratories and with different methods diminished ceruloplasmin levels were found in patients with Wilson's disease The first method used was based on its enzymatic activity [43] as described by Holmberg and Laurell and the second more elegant method based on quantitative immunologic procedures in which antiserum had been prepared against crystalline ceruloplasmin [22] A colorimetric procedure based on its oxidase properties has also been employed

Although the majority of patients with Wilson's disease have a diminished serum ceruloplasmin level the degree of depression varies widely In some individuals essentially no ceruloplasmin can be detected In others the level is depressed to about 25 per cent of its normal value (Fig 25-1) Several sibships with Wilson's disease have been reported in whom the ceruloplasmin levels were within the normal range Whether the ceruloplasmin present is precisely the same molecule which exists in normal subjects requires further study

In some patients with Wilson's disease as in normal subjects an increase in the ceruloplasmin concentration can be achieved by the administration of estrogens (Fig 25-2) [46] This effect is most marked in those in whom the ceruloplasmin level is least depressed As might be anticipated in those patients in whom little or no ceruloplasmin can be detected no measurable increase in the ceruloplasmin level occurs In some patients a pronounced cupruresis may follow the administration of estrogens [46a]

tributed in all parts of the brain The selective deposition of copper in the basal ganglia has been unduly stressed Vogel [35a] has recently demonstrated an increased copper content of the brain of goldfish when reared in an environment in which the ionic copper is experimentally increased

Ceruloplasmin in trace amounts has been found in the liver and the kidney [36] The physiologic significance of this observation if any is uncertain

*Secretions*

Copper is present in trace amounts in most of the body fluids including saliva milk tears and sweat The urine in the absence of significant proteinuria contains only small amounts of copper the daily 24 hr urine excretion rarely exceeding 0 1 mg per day The form in which copper is present in normal urine is uncertain only part is freely dialyzable

*Physiologic Variations in Copper Metabolism*

*The significance of the alterations in copper metabolism in Wilson s* disease cannot be appreciated unless the normal physiologic variations are understood

Ceruloplasmin increases under a variety of physiologic conditions It may be increased in pregnancy infections thyrotoxicosis cancer and cirrhosis of the liver It is frequently decreased in the newborn and in sprue nephrosis anemia (in infants) and malnutrition (in infants) It is almost invariably decreased in Wilson s disease The administration of estrogens to normal subjects increases the ceruloplasmin level [37]

## ABNORMALITIES OF COPPER METABOLISM IN WILSON S DISEASE

*Historical*

A number of early German workers implicated various heavy metals including copper as possible etiologic agents in Wilson s disease In 1913 only 2 years after Wilson s classic paper Rumpel reported an increased silver and copper content of the liver and kidneys of patients who had died from pseudosclerosis [38] In retrospect it appears that these patients were almost certainly suffering from unrecognized Wilson s disease *Nine years later Siemerling and Oloff, on the basis of the similarity of* the eye cataracts in Wilson s disease to those following injury from a copper containing foreign body made the bold suggestion that copper might be deposited in the liver and brain in this disease as well as in the eye and might be directly responsible for the disease [39]

Because of technical difficulties the possible role of heavy metals in the etiology of the disease remained confused until 1930 when Haurowitz convincingly demonstrated an increased copper content of the liver and brain [40] Despite this unequivocal clue the subject lay dormant until

1945 when Glazebrook reported a patient with Wilson s disease whose liver and brain at autopsy revealed an increased copper concentration and whose serum copper was considered elevated [41] (In view of subsequent findings the increased serum copper reported may possibly have been in error ) In all events the observations of Glazebrook proved to have catalytic consequences and a bewildering spate of papers on copper metabolism ensued The next major event was the chance finding by Mandelbrote and his coworkers [42] that a patient suffering from Wilson s disease excreted an increased quantity of copper in the urine Numerous observers have now amply confirmed both the increased tissue copper and the high urinary copper excretion Considerably more disagreement has developed in regard to the serum copper concentration in Wilson s disease Although initial observers reported both normal and elevated levels it was subsequently shown that the levels were almost invariably decreased [43] Recently the wheel has turned full circle and several patients with unequivocal Wilson's disease have now been observed in whom the serum copper is unquestionably normal [44 45] whether this is normal ceruloplasmin will be discussed more fully later

*Serum Ceruloplasmin*

Almost simultaneously in different laboratories and with different methods diminished ceruloplasmin levels were found in patients with Wilson s disease The first method used was based on its enzymatic activity [43] as described by Holmberg and Laurell and the second more elegant method based on quantitative immunologic procedures in which antiserum had been prepared against crystalline ceruloplasmin [22] A colorimetric procedure based on its oxidase properties has also been employed

Although the majority of patients with Wilson s disease have a diminished serum ceruloplasmin level the degree of depression varies widely In some individuals essentially no ceruloplasmin can be detected In others the level is depressed to about 25 per cent of its normal value (Fig 25 1) Several sibships with Wilson s disease have been reported in whom the ceruloplasmin levels were within the normal range Whether the ceruloplasmin present is precisely the same molecule which exists in normal subjects requires further study

In some patients with Wilson s disease as in normal subjects an increase in the ceruloplasmin concentration can be achieved by the administration of estrogens (Fig 25 2) [46] This effect is most marked in those in whom the ceruloplasmin level is least depressed As might be anticipated in those patients in whom little or no ceruloplasmin can be detected no measurable increase in the ceruloplasmin level occurs In some patients a pronounced cupuresis may follow the administration of estrogens [46a]

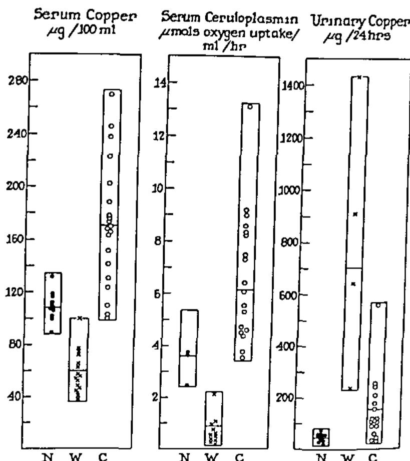



Fig 25-1 Comparison of amounts of serum copper serum ceruloplasmin and urinary copper in normal subject cirrhotic patients and patients with Wilson s disease Horizontal lines represent mean values Note the one patient with Wilson s disease in whom a normal ceruloplasmin level occurred (*Reproduced from A G Bearn [69]*)

*Serum Copper*

Early in the course of investigations on Wilson s disease it was found that the serum copper level was higher than would have been expected from the ceruloplasmin levels observed Since there was no evidence that ceruloplasmin in Wilson s disease contained more copper per molecule it seemed probable that the nonceruloplasmin copper was increased Observations by Cartwright have now established that the direct reacting copper is elevated in Wilson s disease (Fig 25 3) It is possible

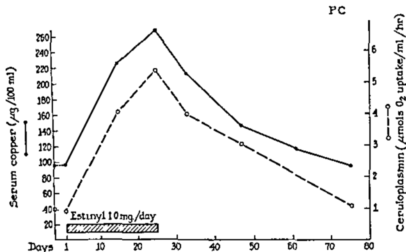



Fig 25-2 Effe t of estinyl on the serum ceruloplasmin of a patient with W Lon s d case (*Pep o l ce l from A G Bearn [ 0] with pers i on of the Ho iorary Fd t s of the Royal Society of M d cine*)

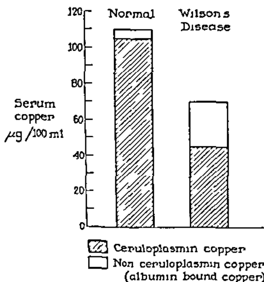



Fig 25 3 Note (1) d crease in total serum copp r (2) d cr ase in serum c rulo plasmin (3) increase in dire t reacting copper (albumin bound copper) in Wilson s disease

that the increased concentration of the direct reacting copper reflects the mechanism by which excess copper is deposited in the tissue

*Urinary Copper Excretion*

Although an increased copper excretion is usually considered to be one of the cardinal biochemical abnormalities in Wilson's disease it should be appreciated that a moderate increase in urinary copper excretion can occur in cirrhosis of the liver particularly of the biliary type Urinary copper excretion estimations are, therefore unreliable in excluding Wilson's disease as a cause for juvenile cirrhosis Fortunately this important clinical differential diagnosis can usually be settled by a determination of the level of serum ceruloplasmin (Fig 25 1)

The extent of the increased urinary copper excretion in Wilson s disease varies considerably In general those individuals with the shortest duration of clinical disease tend to have the more nearly normal levels, those in whom the disease has been present for a long time may excrete as much as 1 5 mg copper daily In individual patients it is usually possible to observe a slow but progressive increase in the urinary excretion of copper over a period of years Thus far no patient with clear clinical evidence of Wilson's disease has been seen in whom the urinary copper excretion was completely normal

Under standard dietary conditions day to-day fluctuations in the urinary excretion of copper are relatively slight and are largely independent of the urinary volume and the dietary copper intake [47] In contrast to the insensitivity of the urinary excretion of copper to a twenty five fold increase in copper intake a ten fold increase in the dietary protein intake results in an approximately two fold increase in the urinary excretion of copper In normal subjects trivial and inconstant increases in copper excretion are observed following a similar increase in the protein intake The increased copper excretion which follows the augmented protein intake is paralleled by the increased amino acid excretion It is tempting to ascribe in part the increased copper excretion to the capacity of many amino acids to chelate copper However quantitative considerations compel one to qualify such an interpretation for chelation implies a necessary molar relationship between the quantity of amino acids and the quantity of copper excreted

*Tissue Copper*

The intrahepatic distribution of copper in Wilson s disease has been recently studied by Uzman using a histologic method [48] Rubeanic acid forms a black precipitate with copper possibly because of the formation of the imido internal salt of rubeanic acid Under neutral or slightly alkaline conditions this reaction has a reported sensitivity of 0 006 $\mu g$ copper The original method using rubeanic acid suffered from certain

drawbacks The modification recently introduced appears more satisfactory although attention to detail is still imperative if reproducible results are to be obtained A marked variation in the copper content of various liver lobules has been described

The intracellular distribution of copper can be arbitrarily divided into three stages At first a fine diffuse distribution of copper is present throughout the cytoplasm of the liver cell Later copper is concentrated around one pole of the nucleus In the heavily involved lobules the granular copper deposits near the nucleus are coarse but become finer as the periphery of the cell is approached Copper is not found in the Kupffer cells of the liver Examination of the histologic appearance of the liver at various stages of the disease suggests that the copper deposition precedes the cirrhotic process It seems clear that the liver cells have a particular affinity for copper Whether this is because of the presence of specific abnormal liver proteins with a high affinity for copper or because of the binding of copper to normal cellular constituents will be discussed in detail later

In the brain copper is distributed widely No single area appears exempt In some instances copper is deposited in large quantities in the cerebral cortex, but usually the basal ganglia bear the brunt of the disease Although the details of the histologic localization of copper in the brain remain uncertain its presence in glial rather than nerve cells has been reported The neurons however show deterioration [49]

The Kayser Fleischer ring has been examined for the presence of heavy metals ever since the early studies of Siemerling and Orloff Recently the histochemical and electron microscopic studies of Uzman have clarified a rather confused subject [50] It has now been demonstrated by two groups of workers that the copper content of the substantia propria may be increased without causing any cloudiness of the cornea or any noticeable color change The copper in the substantia propria is alcohol insoluble but easily removed by dilute acids or chelating agents such as Versene In Descemet s membrane on the other hand the copper is localized as a fine granular deposit arranged in two parallel zones close to the endothelial surface of the membrane The characteristic brown or gray green color of the Kayser Fleischer ring is not directly due to the deposition of copper in Descemet s membrane The presence of dense layers of copper depositions separated by intervening clear zones provides an ideal physical system for the scattering and reflection of incident light on the cornea and gives rise to the characteristic appearance of the Kayser Fleischer ring

### *Dynamic Aspects of Copper Metabolism*

Thus far this account of the abnormalities of copper metabolism in Wilson s disease has been entirely restricted to the realm of descriptive

biology, it seems appropriate now to consider some of its dynamic aspects This problem has been clarified by the use of radioactive isotopes Copper[64] has a half life of only 12 88 hr which limits the time during which studies on man can be performed However another isotope of copper $Cu^{67}$ with a considerably longer half life exists if it becomes available, it will enable prolonged studies to be performed to supplement the observations obtained using $Cu^{64}$

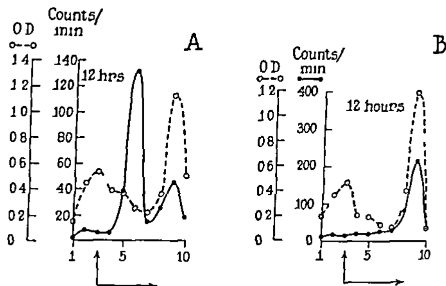



Fig 25-4 Distribution of radioactive copper in the electrophoretically separated fractions of the serum of control subjects (A) and a patient with Wilson s disease (B) 12 hrs following oral administration of $Cu^{64}$ (*Adapted from A G Bearn et al [2]*)

A characteristic and reproducible series of curves is obtained after the intravenous administration of radioactive copper to normal subjects [*21, 91 92*] (Fig 25-4) An initial rapid fall in serum labeled copper is followed by a rise This is succeeded by a slow decline of radioactivity In patients with Wilson s disease the initial decline of radioactivity is slower than in normal subjects and no secondary rise is observed Electrophoretic separation of serum and salt fractionation at various intervals after administration of the isotope have shown that immediately following the administration of $Cu^{64}$ the radioactivity is bound to serum albumin and no radioactivity is detected in any of the other serum fractions During the normal secondary rise the radioactivity is associated with the ceruloplasmin and little or no labeled copper is found in association with the albumin In patients with Wilson s disease as in normal subjects the $Cu^{64}$ is first associated with serum albumin but it remains bound to albumin at a time when in normal persons the $Cu^{64}$ is associated with ceruloplasmin (Fig 25 5)

The tentative explanation of these findings is that in both normal subjects and patients with Wilson s disease copper is transported on the serum albumin to various parts of the body Some copper is transported to the site of ceruloplasmin synthesis where provided the capacity to synthesize ceruloplasmin is normal it becomes incorporated into newly synthesized ceruloplasmin Since impaired ceruloplasmin synthesis is a usual feature of Wilson s disease the copper transported to the liver is not utilized in this way it is deposited at various tissue sites and remains

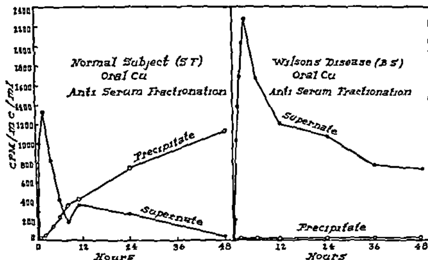



Fig 25 5 Radioactivity in fractions of plasma produced by addition of rabbit anti human ceruloplasmin serum The values on the ordinate refer to counts per minute per milli ure radio opper ingeste l appearing in fractions derived from 1 ml plasma (R prod (from J I Bu h et al (5 ))

bound to the albumin in quantities greater than in normal subjects The copper albumin complex is a relatively loose bond the copper can easily be dissociated from the protein moiety The increased quantity of copper in the urine in Wilson s di ease is no longer anomalous for although the total circulating copper is dimini hed the copper bound to serum albumin is increased and copper is excreted in the urine An asymptomatic normal sibling in whom ceruloplasmin was deficient handled intravenous $Cu^{64}$ similarly to patients with clinical Wilson s disease [58]

Recently Sass Kortsak has studied the fate of intravenous $Cu^{64}$ in a patient with Wilson s disease in whom the ceruloplasmin level was normal [45] No radioactivity could be demonstrated in association with the ceruloplasmin and the $Cu^{64}$ remained associated with the serum albumin as in those patients with Wilson s disease in whom ceruloplasmin was depressed Whether this failure of incorporation of $Cu^{64}$ into cerulo-

plasmin is evidence of a failure to exchange the radioactive copper with the nonradioactive copper or whether as seems more likely, it is due to *an alteration in the structure of ceruloplasmin*, remains to be determined

Since the total body copper is increased in Wilson's disease and because the urinary excretion is elevated it is clear that an increased quantity of dietary copper must be absorbed through the intestinal tract Balance studies have tended to indicate that increased absorption does indeed occur as does a decreased excretion of copper through the intestinal tract The use of radioactive copper has enabled the increased absorption of copper to be demonstrated clearly *[52]*

Uptake of $Cu^{64}$ by erythrocytes (in erythrocuprein) of patients with Wilson s disease is similar to that of normal subjects This emphasizes the rather specific nature of the ceruloplasmin defect in this condition *[52]* An explanation for the hemolytic anemia found in some patients with Wilson s disease is not readily deduced from the studies on copper metabolism of the red cell *[54]*

## RENAL ABNORMALITIES IN WILSON'S DISEASE

In 1948 Uzman and Denny Brown reported an increased amino aciduria in patients with Wilson s disease *[55]* Dent in the previous year had *also observed an increased excretion of amino acids in Wilson s dis* ease *[56]* In the succeeding 10 years it has become clear that the renal lesion in Wilson s disease is extremely diffuse and affects many aspects of renal function *[57]*

### *Renal Hemodynamics*

In most cases of Wilson s disease there is a marked and consistent reduction of renal plasma flow and a distinctly decreased glomerular filtration rate In general the degree of impairment parallels the severity and duration of overt di case In some instances albuminuria can be demonstrated There may be impairment in urine concentrating ability

### *Tubular Reabsorptive Mechanisms*

The tubular secretory capacity is usually diminished as judged by impairment of the $Tm_{PAH}$ Associated with this defective function a diminished effect of probenecid in eliciting a further reduction of $Tm_{PAH}$ can be demonstrated

### *Amino Acids*

Impairment of renal tubular reabsorptive activities in Wilson s di ease has been thoroughly demonstrated The excessive *aminoaciduria* can be attributed to a defective renal reabsorption since qualitatively and quantitatively the amino acids in the serum are not elevated It is known

that the various amino acids are not reabsorbed in the tubules by a common process thus it is of interest to examine the distribution of the amino acids excreted in the urine in patients with Wilson's disease. It should be emphasized that increased aminoaciduria is not a constant feature of the disease and is usually present only in those patients in whom the disease is of long standing. In general it appears that the largest excretion relative to normal subjects occurs with threonine and cystine excretion of which may be elevated twentyfold [58]. Indeed the excretion of cystine in Wilson's disease may exceed that found in patients

TABLE 25-3 URINARY AMINO ACID EXCRETION IN WILSON'S DISEASE

| Diminished | Normal | Increased (× normal) 2–4 | 5–10 | >10 | Excretion of amino acids not found in normal urine |
|---|---|---|---|---|---|
| Taurine | Aminoadipic acid | Histidine | Serine | Threonine | Proline |
| 1 Methyl histidine | Methionine | Ornithine | Glycine | Cystine | Citrulline |
| 3 Methyl histidine | Isoleucine | Phenylalanine | Asparagine | | |
| | Leucine | | Glutamine | | |
| | Arginine | | Valine | | |
| | | | Tyrosine | | |
| | | | Lysine | | |

SOURCE W H Stein et al [58]

with cystinuria. It is of interest that patients with Wilson's disease usually do not form cystine stones. The excretion of other amino acids in relation to the normal excretion is illustrated in Table 25-3.

The urinary excretion of amino acids in Wilson's disease can vary markedly depending upon the state of the disease and the composition of the diet.

In addition to an increased excretion of free amino acids there is an increased excretion of amino acids in the form of conjugated linkages from which the free acids are liberated by hydrolysis with acid. These bound amino acids excreted in quantities about double those of the normal urine thus represent a smaller proportional elevation than that found for free amino acids [58].

In recent years Uzman has emphasized that one of the cardinal abnormalities in Wilson's disease is the excretion of specific oligopeptides [59]. These are thought to be formed by an abnormal proteolytic enzyme in the tissues and to cause an intracellular accumulation of oligopeptides

plasmin is evidence of a failure to exchange the radioactive copper with the nonradioactive copper, or whether, as seems more likely, it is due to an alteration in the structure of ceruloplasmin, remains to be determined

Since the total body copper is increased in Wilson's disease and because the urinary excretion is elevated, it is clear that an increased quantity of dietary copper must be absorbed through the intestinal tract Balance studies have tended to indicate that increased absorption does indeed occur, as does a decreased excretion of copper through the intestinal tract The use of radioactive copper has enabled the increased absorption of copper to be demonstrated clearly [52]

Uptake of $Cu^{64}$ by erythrocytes (in erythrocuprein) of patients with Wilson's disease is similar to that of normal subjects This emphasizes the rather specific nature of the ceruloplasmin defect in this condition [52] An explanation for the hemolytic anemia found in some patients with Wilson's disease is not readily deduced from the studies on copper metabolism of the red cell [54]

## RENAL ABNORMALITIES IN WILSON'S DISEASE

In 1948 Uzman and Denny Brown reported an increased amino aciduria in patients with Wilson's disease [55] Dent in the previous year had also observed an increased excretion of amino acids in Wilson's disease [56] In the succeeding 10 years it has become clear that the renal lesion in Wilson's disease is extremely diffuse and affects many aspects of renal function [57]

### *Renal Hemodynamics*

In most cases of Wilson's disease there is a marked and consistent reduction of renal plasma flow and a distinctly decreased glomerular filtration rate In general the degree of impairment parallels the severity and duration of overt disease In some instances albuminuria can be demonstrated There may be impairment in urine concentrating ability

### *Tubular Reabsorptive Mechanisms*

The tubular secretory capacity is usually diminished as judged by impairment of the $Tm_{PAH}$ Associated with this defective function a diminished effect of probenecid in eliciting a further reduction of $Tm_{PAH}$ can be demonstrated

### *Amino Acids*

Impairment of renal tubular reabsorptive activities in Wilson's disease has been thoroughly demonstrated The excessive aminoaciduria can be attributed to a defective renal reabsorption since qualitatively and quantitatively the amino acids in the serum are not elevated It is known

that the various amino acids are not reabsorbed in the tubules by a common process thus it is of interest to examine the distribution of the amino acids excreted in the urine in patients with Wilson s disease It should be emphasized that increased aminoaciduria is not a constant feature of the disease and is usually present only in those patients in whom the disease is of long standing In general it appears that the largest excretion relative to normal subjects occurs with threonine and cystine excretion of which may be elevated twentyfold [58] Indeed the excretion of cystine in Wilson s disease may exceed that found in patients

TABLE 25-3 URINARY AMINO ACID EXCRETION IN WILSON S DISEASE

| *Diminished* | *Normal* | *Increased (× normal)* | | | *Excretion of amino acids not found in normal urine* |
|---|---|---|---|---|---|
| | | 2–4 | 5–10 | >10 | |
| Taurine | Aminoadipic acid | Histidine | Serine | Threonine | Proline |
| 1 Methyl histidine | Methionine | Ornithine | Glycine | Cystine | Citrulline |
| 3-Methyl histidine | Isoleucine | Phenylalanine | Asparagine | | |
| | Leucine | | Glutamine | | |
| | Arginine | | Valine | | |
| | | | Tyrosine | | |
| | | | Lysine | | |

SOURCE W H Stein et al [58]

with cystinuria It is of interest that patients with Wilson s disease usually do not form cystine stones The excretion of other amino acids in relation to the normal excretion is illustrated in Table 25-3

The urinary excretion of amino acids in Wilson s disease can vary markedly depending upon the state of the disease and the composition of the diet

In addition to an increased excretion of free amino acids there is an increased excretion of amino acids in the form of conjugated linkages from which the free acids are liberated by hydrolysis with acid These bound amino acids excreted in quantities about double those of the normal urine thus represent a smaller proportional elevation than that found for free amino acids [58]

In recent years Uzman has emphasized that one of the cardinal abnormalities in Wilson s disease is the excretion of specific oligopeptides [59] These are thought to be formed by an abnormal proteolytic enzyme in the tissues and to cause an intracellular accumulation of oligopeptides

within the tissues Some of the excreted peptides compete with the amino acids for reabsorption by the proximal renal tubules and, because the copper peptide complex is absorbed preferentially an aminoaciduria results In discussions of this hypothesis it should be recalled that the liberation of amino acids on acid hydrolysis of urine is not evidence per se of the existence of peptides At least 75 per cent of the amino acids liberated by acid hydrolysis of human urine derives from compounds other than peptides [60] A considerable proportion of the increase in the amounts of glutamic and aspartic acids after acid hydrolysis arises from the decomposition of glutamine and asparagine Other compounds include such substances as hippuric acid phenacetyl glutamine and tyrosine O sulfate However small quantities of peptides undoubtedly exist in normal urine and may be presumed to exist in the urine of patients with Wilson's disease Unequivocal demonstration of their presence will be awaited with interest

### *Glucose*

Glycosuria may be present in some patients with Wilson s disease It is significant that although in many patients spontaneous glycosuria may be absent or minimal the maximum tubular capacity to reabsorb glucose is frequently substantially reduced [57] These observations emphasize that a failure to find an increased excretion of glucose does not preclude a considerable defect in the capacity of the renal tubules to transport glucose across the tubular epithelium

### *Bicarbonate*

A tendency to excrete an alkaline urine in patients with Wilson s disease has been described and is associated with the renal excretion of bicarbonates at plasma levels that would ordinarily demand complete reabsorption of bicarbonate by the renal tubules

### *Uric Acid*

In 1954 Bishop and coworkers reported a low serum uric acid associated with an increased urinary uric acid excretion in a brother and sister with Wilson s disease [61] Subsequent investigators have confirmed these findings and renal clearance studies have demonstrated that as originally postulated the diminished reabsorption of filtered urate accounts for both the increased urinary excretion and the low serum urate levels observed The capacity of probenecid further to increase the $C_{urate}$ to $C$ [illegible] diminished as the tubular transport systems for urate deteriorated with progression of the disease [57] The low serum uric acid level is so constant a feature of Wilson s disease that a serum uric acid determination should be performed in all cases of cirrhosis of unexplained origin and in all obscure diseases of the central nerv

ous system in which disturbances of the function of the basal ganglia are prominent

*Phosphate*

A low serum inorganic phosphate level occurs in a high proportion of patients with Wilson s disease [57] Renal clearance studies have revealed an even higher proportion of patients in whom the $C_{Po}$ is increased Thus



Fig 25-6 Radiograph illustrating fracture head of the right radius showing no evidence of bone union (*Reproduced from A G Bearn [76]*)

a failure of normal tubular transport of phosphate is a common feature of the disease and may be in part responsible for the occasional appearance of clinical osteomalacia and spontaneous fractures (Fig 25-6) [*62 63*]

*Calcium*

Hypercalcinuria occurs in many patients with Wilson s disease and is also related in part to the duration of the overt disease [*63a*]

## THE ABNORMALITY IN WILSON S DISEASE

In the preceding pages the various abnormalities in copper metabolism and renal function have been discussed and the two current hypotheses of the pathogenesis of the disease briefly mentioned These two hypotheses will now be discussed in detail

*Abnormality of Copper Metabolism*

In its simplest form this hypothesis suggests that the primary effect of the abnormal gene is to diminish the synthesis of normal ceruloplasmin

and conceivably of other closely related proteins In a way which is not understood this leads to an increased absorption of copper from the intestinal tract, and this in turn to an excessive deposition of copper in the tissues with consequent functional and structural alterations Although the copper content of many organs is increased certain tissues such as the basal ganglia of the brain, liver cells and Descemet's membrane of the cornea appear to be selected preferentially The differential deposition of copper in various tissues could be ascribed to the variation of copper binding capacity of *normal* proteins and related substances in different tissues No postulation that these tissue proteins are abnormal would be necessary Thus the disturbed liver function leading eventually to cirrhosis of the liver the cerebral damage, and the disturbance in renal function are all ultimately consequent upon the effect of copper on a variety of enzyme systems throughout the body The decreased ceruloplasmin level observed in some individuals who are heterozygous indicates that even in single dose the abnormal gene may have an observable metabolic defect but without causing manifest clinical disease

When this hypothesis is stated in this simple way many experimental observations do not fit (1) There is no correlation between the depression of ceruloplasmin synthesis and the amount of copper absorbed (2) Experimental administration of copper to a variety of animals has failed to mimic the disease (3) Some patients with Wilson s disease have a serum ceruloplasmin level which is quantitatively normal (The direct reacting copper level is usually elevated in these individuals, this probably reflects the increased body stores of copper ) (4) There is relatively little correlation between the increased amount of copper excreted in the urine and the clinical improvement which may follow the use of British anti lewisite (BAL) and penicillamine Many of these objections do not by themselves invalidate this hypothesis but serve to emphasize its considerable defects

A reasonable hypothesis for the renal lesion ascribes the progressive deterioration in renal function to the march of copper to sites where it can interfere with enzyme systems responsible for the transport of materials across epithelial membranes Such a hypothesis gains some support from observations on poisoning with other divalent metals in which most of the damage falls, as it does in Wilson s disease on the proximal renal tubules It seems plausible that the renal abnormalities can be ascribed to a disturbance in copper metabolism, and are not a direct consequence of the abnormal gene on renal function

### *Abnormality of Peptide Metabolism*

An alternative hypothesis has been postulated by Uzman and outlined by him in a series of papers [59 64 65] This hypothesis ascribes the primary effects of the abnormal gene to an abnormal protein which has

an increased capacity for binding ionic copper This protein is present in the liver (and presumably in every tissue in which the copper content is increased) The increased affinity of copper for certain tissues blocks the formation of ceruloplasmin thus the diminished ceruloplasmin level is a secondary phenomenon and is due to the diversion of copper to the tissues In addition the abnormal protein metabolism in the tissues is said to give rise to specific oligopeptides which compete with amino acids and uric acid for reabsorption in the proximal renal tubules, causing aminoaciduria and uricosuria Cirrhosis of the liver is ascribed to a nutritional deficiency

This hypothesis currently less favored than the first also contains some unsatisfactory feature Quantitative considerations make it extremely improbable that the renal loss of amino acids could give rise to hepatic cirrhosis and indeed many cases of Wilson s disease have been reported in which no aminoaciduria is present but in whom a diminished ceruloplasmin is evident [58 66] The difficulties and uncertainties in the peptide hypothesis for the urinary abnormalities have been outlined in an earlier section and have been dealt with at length by Walshe [67] According to this hypothesis it would be anticipated that intravenously administered radioactive copper would disappear from the circulation more rapidly rather than more slowly if the tissue proteins had an increased affinity for copper Thus it can be seen that neither hypothesis can be regarded as more than tentative the problem will be resolved not by debate but by the collection of more data

## GENETICS

In his original monograph Kinnier Wilson pointed out that familial incidence is one of the striking features of the disease Although he considered the possibility that the disease was inherited the familial incidence was attributed to environmental rather than genetic causes [1] This view with some notable dissent [68] was held until relatively recently

Study of a large number of patients with Wilson s disease has permitted a genetic analysis [69 70] Inspection of the pedigrees immediately confirmed the familial concentration observed by Wilson and in addition disclosed that many of those afflicted were the offspring of consanguineous unions (Table 25-4)

This remarkably high consanguinity rate is good evidence that the disease is inherited in an autosomal recessive fashion (Table 25-4) Calculations based on the incidence of Wilson s disease in siblings when the parents are unaffected are in accord with this hypothesis [70] The method of calculation used is variously known as Hogben s factorial method or the a priori method of Bernstein

TABLE 25-4 THE CONSANGUINITY RATE IN 30 FAMILIES WITH WILSON'S DISEASE

| No of sibships | *Consanguinity rate in parents of patients with Wilson's disease* | | | | | |
|---|---|---|---|---|---|---|
| | *First cousins* | | *Second cousins* | | *Unrelated* | |
| | No | Percentage | No | Percentage | No | Percentage |
| 30 | 11 | 36 7 | 3 | 10 0 | 16 | 53 3 |

SOURCE Adapted from A G Bearn [70a]

## *Sex Incidence*

The disease is probably slightly more common in males than females Most series of cases show a slight excess in males which because of the small numbers is statistically insignificant In our series 21 of 32 patients were male

## *Age of Onset*

The age of onset of Wilson's disease is variable and does not differ in the two sexes (Table 25 5) The apparent excess of Jewish males when the

TABLE 25 5 THE ESTIMATED AGE OF ONSET AND GEOGRAPHIC ORIGIN OF 32 PATIENTS WITH WILSON'S DISEASE

| Age of onset yr | Consanguinity | *Sex* | | *Geographic origin* | | | | *Total* |
|---|---|---|---|---|---|---|---|---|
| | | *Male* | *Female* | *Eastern European (Jewish)* | *Mediterranean* | *Negro* | *Other* | |
| 10–24 | R | 5 | 3 | 3 | 3 | 0 | 2 | 8 |
| | U | 5 | 3 | 1 | 2 | 1 | 4 | 8 |
| | T | 10 | 6 | 4 | 5 | 1 | 6 | 16 |
| 25–39 | R | 4 | 4 | 6 | 1 | 1 | 0 | 8 |
| | U | 7 | 1 | 4 | 2 | 0 | 2 | 8 |
| | T | 11 | 5 | 10 | 3 | 1 | - | 16 |

Includes one sibling pair
Note R = related U = unrelated T = total
SOURCE Adapted from A G Bearn [70a]

disease has a late onset raises the problem of genetic heterogeneity The possibility exists that the allele causing disease in this group of patients is different from the usual allele A modifying gene which delays the onset of the disease could also be responsible for the late onset in these patients

*Racial Incidence*

Of the 30 families in New York City in whom individuals with Wilson's disease were discovered 13 (43 per cent) were Jewish who came from the border of Russia and Poland within a radius of approximately 100 miles. If a mutation occurred in this population the breeding structure would favor the formation of many homozygotes. Seven of the 30 families (23 per cent) were Italians who came from Sicily and the southernmost tip of Italy. The breeding structure in this isolated area would also tend to produce individuals homozygous for the abnormal gene. Nearly all the remainder of the patients of this series came from various parts of Europe. Two of the 30 families (7%) were Negroes. The disease has also been recorded in Chinese, Malayans, Japanese and Eskimos [70b]. Whether the original mutations which occurred in Eastern Poland and Sicily were identical is not clear. In interpreting these data it must be recalled that these patients were largely collected from New York City and that their racial distribution reflects to some extent the geographic origins of the population of New York.

*Gene Frequency*

Estimations of gene frequency are fraught with considerable hazard. One of the essential assumptions, namely that the disease is genetically homogeneous, may be unjustified. Another difficulty encountered if the method devised by Dahlberg [71] is used is the uncertainty of the estimate of the frequency of first cousin marriage in the population from which the sample is drawn. Few precise data are available concerning the estimate of the first cousin consanguinity rate in the populations of Eastern Poland or Sicily 50 years ago. To be sure the figure of 0.006 estimated by Julia Bell [72] for England is far too low and would give too low an estimate of the gene frequency. If accurate data on the consanguinity rate were known it would be possible to give a rough estimate of the gene frequency in the original populations in Eastern Poland and Sicily. It is impossible, however, to determine the gene frequency in America with any accuracy. As a very rough estimate if the consanguinity rate in America is assumed to be 0.005 the calculated gene frequency would be approximately 1/2,000 with a disease incidence of 1/4,000,000. It should be emphasized that such calculations are almost valueless in a rare recessive disease in which the condition may have arisen by mutation in a very small community in which consanguineous unions were very numerous.

*Detection of Carriers*

The detection of asymptomatic carriers of deleterious genetic traits is being attempted in many heritable disorders. Related to this problem

but frequently more difficult to demonstrate is the important question of whether the heterozygote possesses any increased biologic fitness over either homozygote [73] Unfortunately, with a disease as rare as Wilson's disease it would be virtually impossible to demonstrate any heterozygous advantage

If the gene product were known with certainty, then its estimation should enable a differentiation between the three genetic groups—homozygous normal, homozygous affected and heterozygous—although some degree of overlap might be expected Ceruloplasmin depression a relatively constant biochemical abnormality in patients with Wilson's disease has been found in some heterozygotes, and not in others [70 74 75] In addition some asymptomatic siblings without Kayser Fleischer rings have had a low level of ceruloplasmin and were found unable to incorporate $Cu^{64}$ into ceruloplasmin [45] A preliminary study of 10 heterozygotes with normal ceruloplasmin levels has demonstrated less incorporation of ingested $Cu^{64}$ into ceruloplasmin than normal [76] The possibility that aminoaciduria could be detected in asymptomatic siblings of patients with Wilson's disease has been investigated The first reports indicated that aminoaciduria could be detected in relatives of those affected [51] Subsequent work has failed to confirm the early findings [47]

There is evidence that the disease in certain families conforms to a distinctive clinical and biochemical pattern In any one sibship the age of onset, the predominant clinical symptoms, and the biochemical abnormalities may be remarkably similar In some families only the hepatic form of the disease is present, in others the liver injury is minimal and neurologic symptoms are prominent The possibility cannot be dismissed that these two clinical syndromes are due to different alleles which may not even be at the same locus [70]

Muller has recognized two categories of gene products which he has called amorphs and neomorphs The first is associated with an absence of the gene product and the latter with the formation of a new and altered gene product Wilson's disease may prove to be an example of a disease which is amorphic (usually hypomorphic) but which may occasionally prove to be neomorphic

## TREATMENT

Until some of the biochemical features outlined in the preceding pages became delineated the treatment of Wilson's disease was entirely symptomatic On the assumption that some if not all of the symptoms in Wilson's disease may be related to the increased total body copper measures designed to decrease the tissue copper stores might be expected to be therapeutically beneficial

It is not possible to reduce the copper content of the diet below 1 0 mg per day without considerable caloric restriction The ingestion of carbo

resins and potassium sulfide has been advocated to reduce copper absorption

In addition to diminishing the copper intake efforts should be made to achieve a large and persistent increase in urinary copper excretion A variety of agents which chelate copper have been used with success BAL introduced by Denny Brown [77] has proved a valuable agent in increasing the urinary excretion of copper It suffers from the disadvantage that it must be suspended in peanut oil and administered by intramuscular injection Local induration or abscesses may occur at the site of injection Moreover toxic reactions to BAL are not uncommon These include high fever faintness nausea and vomiting Intravenous dicalcium disodium versenate is an effective cupruric agent but the necessity for intravenous injection of this compound precludes its continued use

The introduction of penicillamine ($\beta$ $\beta$ dimethylcysteine) in 1956 by Walshe was a landmark in the development of therapeutic measures for increasing the urinary excretion of copper [78] This compound which can be given by mouth is in the practical dosage range a much more effective cupruric agent than BAL and its administration is not accompanied by so many side reactions Toxic reactions may occur however and resemble those seen in penicillin sensitivity Fever various drug rashes and leukopenia have been observed Pharmacologic studies of the two enantiomorphs—D penicillamine and L-penicillamine—indicate not only that the D form is the more effective in protecting rats against mercury poisoning but that the L-form has serious toxicity for the animals [79] Such studies do not argue strongly in favor of clinical use of the D-optical isomer rather than the DL-mixture but indicate the need for a comparative therapeutic evaluation

One of the difficulties encountered in the treatment of Wilson s disease has been the tendency of patients to become resistant to the cupruric agent and a prolonged cupruric effect may be difficult to achieve Presumably the copper easily removed is that recently deposited whereas the fraction of copper corresponding to cerebrocuprein II is less easily chelated None of the chelating agents consistently lowers the serum ceruloplasmin level The excretion of amino acids or other substances absorbed by the proximal renal tubules and excreted in excess in Wilson s disease is not affected by the administration of BAL or penicillamine

Varying combinations of these therapeutic measures have been found beneficial in management of patients with this disease The optimum program of administration of the chelating agents has not been clearly defined The natural course of the disease though more often inexorably downhill if untreated may be prolonged and subject even to periods of improvement This makes evaluation of therapy difficult Dramatic improvement sometimes sustained for long periods may follow the use of penicillamine or BAL

## SUMMARY

1 Wilson s disease (hepatolenticular degeneration) is a rare inherited disease which usually occurs in young people Pathologically it is characterized by cirrhosis of the liver and degenerative changes in the brain particularly in the basal ganglia Kayser Fleischer corneal rings are pathognomonic of the disease An increased absorption of dietary copper *from the intestinal tract with consequent increased deposition of copper* in the tissues can be demonstrated The increased copper in the body is localized primarily in the liver brain kidneys and cornea The administration of radioactive copper can increase the number of heterozygotes in whom abnormalities in ceruloplasmin can be detected

2 Biochemical abnormalities in Wilson s disease are multiple Low serum copper and decreased serum ceruloplasmin levels are observed in the majority of patients with the disease and are associated with an increased urinary excretion of copper The plasma levels of amino acids and glucose are usually normal or slightly decreased The serum phosphate level is often decreased and the serum uric acid usually decreased

3 Abnormalities in renal function are demonstrable they consist of a progressive failure of tubular transfer mechanisms for amino acids glucose, uric acid and phosphate

4 Family studies indicate an autosomal recessive inheritance pattern Decreased serum copper and serum ceruloplasmin are occasionally found in clinically normal heterozygotes

5 The pathogenesis of the disease is obscure Whether it is a general abnormality of protein metabolism in which specific tissue proteins bind copper preferentially or whether as seems more likely the primary disturbance is one of copper metabolism remains to be determined The abnormality usually results in a decreased synthesis of normal ceruloplasmin However the possibility that synthesis of an abnormal ceruloplasmin may perhaps occur must be considered

## BIBLIOGRAPHY


1 Wilson S A K Progressive lenticular degeneration a familial nervous disease associated with cirrhosis of the liver Brain **34** 29[illegible] 1912

2 Gower W R On tetanoid chorea and its association with cirrhosis of the liver *Rev Neurol & Psychiat* 9 249 1906

3 Frerichs F T *Pathologisch-anatomischer Atlas zur Klinik der Leberkrankheiten* vol 2 p 62 F Vieweg Braunschweig 1861

4 Westphal C Über eine dem Bilde der cerebro spinalen grauen Degeneration ähnliche *Erkrankung des centralen Nervensystems ohne anatomischen* Befund nebst einigen Bemerkungen uber paradoxe Contraction Arch Psychiatr **14** 87 1883

5 Franklin E C and Bauman A Liver dysfunction in hepatolenticular degeneration a review of *eleven cases Am J Med 15 4[illegible]0 1953*

6 Fleischer B Zwei weitere Falle von grunlicher Verfarbung der Kornea Klin Monat bl Augenh **41** 489 1903

7 Fleischer B Die periphere braungrünliche Hornhautverf rbung als Symptom einer eigenartigen Allgemeinerkrankung Munchen med Wchnschr 56 1120 1909
8 Fleischer B Über eine der Pseudosklerose nahestehende bisher unbekannte Krankheit (gekennzeichnet durch Tremor p ychische Storungen braunliche Pigmentierung bestimmter Gewebe insbesondere auch der Hornhautperipherie Lebercirrhose) Deutsche Ztschr Nervenh 44 179 1912
9 Kehrer F Zur Ätiologie und Nosologie der Pseudosklerose Westphal Wilson Ztschr ges Neurol u Psychiat 129 488 1930
10 Chalmers T C Iber F L and Uzman L L Hepatolenticular degeneration (Wilson s disease) as a form of idiopathic cirrhosis New England J Med 256 235 1957
11 Holmberg C G and Laurell C B Investigations in serum copper II Isolation of the copper containing protein and a description of some of its properties Acta chem scandinav 2 550 1948
12 Schultze H E Gollner I Heide K Schonenberger M and Schwick G Zur Kenntnis der α Globuline des menschlichen Normalserums Z Naturforsch 10b 463 1955
13 Holmberg C G and Laurell C B Oxidase reactions in human plasma caused by ceruloplasmin Scandinav J Clin & Lab Invest 3 103 1951
14 Holmberg C G and Laurell C B Investigations in serum copper IV Effect of different anions on the enzymatic activity of caeruloplasmin Acta chem scandinav 5 9 1 1951
15 Curzon G The chemistry and biochemistry of caeruloplasmin Proc Roy Soc Med 52 64 1959
16 Morell A G and Scheinberg I H Preparation of an apoprotein from ceruloplasmin by reversible dissociation of copper Science 127 588 1958
17 Scheinberg I H and Morell A G Exchange of ceruloplasmin copper with ionic Cu with reference to Wilson s disease J Clin Invest 36 1193 1957
18 Akerfeldt S Oxidation of N N-dimethyl p-phenylened amine by serum from patients with mental disease Science 125 117 1957
19 Scheinberg I H Morell A G Harris R S and Berger A Concentration of ceruloplasmin in plasma of schizophrenic patients Science 126 925 1957
20 Ravin H A Rapid test for hepatolenticular degeneration Lancet 1 726 1956
21 Scheinberg I H Harris R S Morell A G and Dubin D Some aspects of the relation of ceruloplasmin to Wilson s disease Neurology 8 Suppl 1 44 1958
22 Scheinberg I H and Gitlin D Deficiency of ceruloplasmin in patients with hepatolenticular degeneration (Wilson s disease) Science 116 484 1952
23 Broman L Separation and characterization of two coeruloplasmins from human serum Nature 182 1655 1958
23a Sa kort ak A Jackson S J and Charles A F Studies on ceruloplasmin Vox Sanguinis (in press)
24 Bearn A G and Kunkel H G Localization of Cu[64] in serum fractions following oral administration an alteration in Wilson s disease Proc Soc Exper Biol & Med 85 44 1954
25 Fie H A and Klotz I M The thermodynamics of metalloprotein combinations Comparison of copper complexes with natural proteins J Am Chem Soc 74 88 1952
26 Cubler C J Lahey M E Cartwright G E and Wintrobe M M Studies on copper metabolism X The transportation of copper in blood J Clin Invest 32 405 1953
27 Cartwright G E Gubler C J and Wintrobe M M The role of copper in erythropoiesis in Conference on Hemoglobin May 2 3 1957 pp 100–110 National Academy of Sciences—National Research Council Washington D C 1958
28 Kimmel J R Markowitz H and Brown D M Some chemical and physical properties of erythrocuprein J Biol Chem 234 46 1959
29 Markowitz H Cartwright G E and Wintrobe M M Studies on copper

metabolism XXVII The isolation and properties of an erythrocyte cuproprotein (erythrocuprein) J Biol Chem 234 40 1959
30 Gubler C J Copper metabolism in man J A M A 161 530 1956
31 Mann T and Keilin D Haemocuprein and hepatocuprein copper protein compounds of blood and liver in mammals Proc Roy Soc London Ser B *126 303 1938*
32 Mohamed M S and Greenberg D M Isolation of purified copper protein from horse liver J Gen Physiol 37 433 1954
33 Porter H and Folch J Cerebrocuprein I A copper-containing protein isolated from brain J Neurochem 1 260 1957
34 Porter H and Folch J Brain copper protein fractions in the normal and in Wilson s disease A M A Arch Neurol & Psychiat 77 8 1957
35 Porter H and Ainsworth S Reaction of brain copper proteins with sodium diethyldithiocarbamate in normal and in hepatolenticular degeneration Proc Soc Exper Biol & Med 98 277 1958
35a Vogel F S The deposition of exogenous copper under experimental conditions with observations on its neurotoxic and nephrotoxic properties in relation to Wilson s disease J Exper Med 110 801 1959
36 Cartwright G E Hodges R E Gubler C J Mahoney J P Daum K Wintrobe M M and Bean W B Studies on copper metabolism XIII Hepatolenticular degeneration J Clin Invest 33 1487 1954
37 Russ L M and Raymunt J Influence of estrogens on total serum copper and caeruloplasmin Proc Soc Exper Biol & Med 92 465 1956
38 Rumpel A Über das Wesen und die Bedeutung der Leberveränderungen und der Pigmentierungen bei den damit verbundenen Fallen von Pseudosklerose zugleich ein Beitrag zur Lehre von der Pseudosklerose (Westphal-Strumpell) Deutsche Ztschr Nervenh 49 54 1913
39 Siemerling E and Oloff H Pseudosklerose (Westphal Strümpell) mit Corneal ring (Kayser Fleischer) und doppelseitiger Scheinkatarakt die nur bei seitlicher Beleuchtung sichtbar ist und die der nach Verletzung durch Kupfer splitter entstehenden Katarakt ahnlich ist Klin Wchnschr 1 1087 192-
40 Haurowitz F Über eine Anomalie des Kupferstoffwechsels Ztschr physiol Chem 190 72 1930
41 Glazebrook A J Wilson s disease Edinburgh M J 52 83 1945
42 Mandelbrote B M Stanier M W Thompson R H S and Thruston M N Studies on copper metabolism in demyelinating diseases of the central nervous system Brain 71 212 1948
43 Bearn A G and Kunkel H C Biochemical abnormalities in Wilson s disease J Clin Invest 31 616 1952 (abstract)
44 Bearn A G Unpublished observations
45 Sass Kortsak A Cherniak M Geiger D W and Slater R J Observations on ceruloplasmin in Wilson s disease J Clin Invest 38 1672 1959
46 Bearn A G Wilson s disease An inborn error of metabolism with multiple manifestations Am J Med 22 747 1957
47 Bearn A G and Kunkel H G *Abnormalities of copper metabolism in Wilson s* disease and their relationship to the aminoaciduria J Clin Invest 33 400 1954
48 Uzman L L Histochemical localisation of copper with rubeanic acid Lab Invest 5 299 1956
49 Uzman L L in *Proceedings of the Third International Neurochemistry Symposium* Strasbourg 1958 (in press)
50 Uzman L L and Jakus M A The Kayser Fleischer ring a histochemical and electron microscope study Neurology 7 341 1957
51 Bearn A G and Kunkel H C Metabolic studies in Wilson s disease using $Cu^{64}$ J Lab & Clin Med 45 623 1955
52 Bush J A Mahoney J P Markowitz H Gubler C J Cartwright G E and Wintrobe M M Studies on copper metabolism XIV Radioactive copper studies in normal subjects and in patients with hepatolenticular degeneration J Clin Invest 34 1766 1955
53 Bearn A G Unpublished observations

54 Goldberg A Williams C B Jones R S Yanagita M Cartwright G L and Wintrobe M M Studies on copper metabolism XXII Hemolytic anemia in chickens induced by the administration of copper J Lab & Clin Med 48 41 1956
55 Uzman L and Denny Brown D Amino aciduria in hepatolenticular degeneration (Wilson s disease) Am J M Sc 215 599 1948
56 Dent C E Chromatography studies in *Liver Injury Transactions of the 6th Conference on* May 1 2 1947 pp 53 62 Josiah Macy Jr Foundation New York
57 Bearn A G Yu T F and Gutman A B Renal function in Wilson s disease J Clin Invest 36 1107 1957
58 Stein W H Bearn A G and Moore S The amino acid content of the blood and urine in Wilson s disease J Clin Invest 33 410 1954
59 Uzman L L On the relationship of urinary copper excretion to the amino aciduria in Wilson s disease (hepatolenticular degeneration) Am J M Sc 226 645 1953
60 Stein W H A chromatographic investigation of the amino acid constituents of normal urine J Biol Chem 201 45 1953
61 Bishop C Zimdahl W T and Talbott J H Uric acid in two patients with Wilson s disease (hepatolenticular degeneration) Proc Soc Exper Biol & Med 86 440 1954
62 Warnock C G Hepatolenticular degeneration (Wilson s disease) a report of five cases with commentary Ulster M J 21 155 1952
63 Finby N and Bearn A G Roentgenographic abnormalities of the skeletal system in Wilson s disease (hepatolenticular degeneration) Am J Roentgenol 79 603 1958
63a Litin R B Randall R V Goldstein N P Power M H and Diessner G R Hypercalciuria in hepatolenticular degeneration (Wilson s Disease) Am J Med Sci 238 614 1959
64 Uzman L L Iber F L and Chalmers T C Mechanism of copper deposition in the liver in hepatolenticular degeneration (Wilson s disease) Am J M Sc 231 511 1956
65 Iber F L Chalmers T C and Uzman L L Studies of protein metabolism in hepatolenticular degeneration Metabolism 6 388 1957
66 Cooper A M Eckhardt R D Faloon W W and Davidson C S Investigation of the aminoaciduria in Wilson s disease (hepatolenticular degeneration) demonstration of a defect in renal function J Clin Invest 29 265 1950
67 Walshe J M Hepatolenticular degeneration (Wilson s disease) Brit M Bull 13 132 195
68 Matthews W B The absorption and excretion of radiocopper in hepatolenticular degeneration (Wilson s disease) J Neurol Neurosurg & Psychiat New Series 17 24 1954
69 Bearn A G Genetic and biochemical aspects of Wilson s disease Am J Med 15 442 1953
70 Bearn A G Genetic aspects of Wilson s disease Proc Roy Soc Med 52 61 1959
70a Bearn A G A genetical analysis of thirty families with Wilson s disease (hepatolenticular degeneration) Ann Human Genet 24 33 1960
70b Cumings J N *Heavy Metals and the Brain* p 22 Blackwell London 1959
71 Dahlberg G Biometric evaluation of findings in *Clinical Genetics* edited by A Sorsby pp 83–100 Butterworth London 1953
72 Bell J A determination of the consanguinity rate in the general hospital population of England and Wales Ann Eugenics 10 370 1940
73 Penrose L S Quelques principes sur la fréquence des genes et sa stabilité dans les populations humaines J Génét Humaine 3 159 1954
74 Bickel H Neale F C and Hall G A clinical and biochemical study of hepatolenticular degeneration (Wilson s disease) Quart J Med N S 26 527 1957
75 Neale F C and Fischer Williams M Copper metabolism in normal adults

and in clinically normal relatives of patients with Wilson s disease J Clin Path 2 441 1958
76 Sternlieb I Morell A C and Scheinberg I H Detection of the heterozygous carrier of the Wilson s disease gene (abstract) J Clin Invest 38 1046 1959
76a Muller H J Evidence of the precision of genetic adaptation Harvey Lectures Ser 43 p 165 1947–1948
77 Denny Brown D and Porter H The effect of BAL (2 3-dimercaptopropanol) on hepatolenticular degeneration (Wilson s disease) New England J Med 245 917 1951
78 Walshe J M Penicillamine a new oral therapy for Wilson s disease Am J Med 21 487 1956
79 Aposhian H V Protection by D-penicillamine against the lethal effects of mercuric chloride Science 128 93 1958

# Chapter 26

# Hemochromatosis

*Ralph E. Peterson*

Hemochromatosis is a form of iron storage disease characterized by the deposition of abnormally large quantities of iron in various organs or tissues of the body. It was probably first reported as a morbid entity by Troisier in 1871 [1] under the designation *la cirrhose pigmentaire dans le diabete sucré* although Trousseau [2] probably described a case in 1865 in the course of a clinical lecture on glycosuria. The term *hemochromatosis* was first introduced by von Recklinghausen in 1889 [3] to describe the clinical triad of generalized pigmentation, enlarged and cirrhotic liver, and diabetes mellitus. He used the term because he considered the disease an example of a hemorrhagic diathesis. The excess pigment in the tissue was presumed to arise from the breakdown of hemoglobin. The disease has also been referred to as bronzed diabetes, pigment cirrhosis, siderochromatosis, and primary, congenital, idiopathic or endogenous hemochromatosis.

The term *primary hemochromatosis* will be reserved here for the idiopathic form of iron storage disease which in untreated patients eventually progresses to the classical pathologic triad of cirrhosis of the liver, brown discoloration of the skin, and diabetes mellitus. Primary hemochromatosis probably represents an inherited metabolic disorder as first suggested by Sheldon in 1927 [4]. The disease is now known to be associated with an increased gastrointestinal absorption of iron.

## DEFINITIONS

It is necessary to differentiate among the various conditions associated with increased body iron stores (Table 26-1).

Hemochromatosis is the form of tissue iron deposition associated with characteristic morbid changes; may be primary or secondary.

*Primary or idiopathic hemochromatosis* is a specific disease entity

It is one of several forms of iron storage disease known collectively as hemosiderosis (a descriptive histologic term) The increased iron stores in primary hemochromatosis result from increased absorption of the mineral from a normal diet The increased iron absorption is not secondary to any known cause such as increased oral iron intake or erythroid hyperplasia It is believed that this disease represents a genetically determined error of metabolism Primary hemochromatosis usually begins in adult life and is much more common in males than in females

TABLE 40 1 IRON STORAGE DISEASE

| Type | Cause of increased iron stores | Percentage of diet iron absorbed |
|---|---|---|
| Hemochromatosis | | |
| Primary | Inborn error of metabolism | Increased |
| Secondary | Increased erythroid activity | Increased |
| | Excessive oral iron intake | Normal |
| Siderosis | | |
| Localized | Pulmonary hemosiderosis | Normal |
| Generalized | | |
| Transfusion hemosiderosis | Transfused blood | Normal |
| Cytosiderosis (Bantu) | Dietary deficiency plus excess dietary iron | Normal |
| Kashin Beck disease | Increased iron content of drinking water | Normal |

*Secondary hemochromatosis* is a form of siderosis which may result from increased iron absorption secondary to known causes The most common of these is erythroid hyperplasia associated with hemolytic [5] pernicious [6] primary hyperplastic refractory [7–9] or Cooley s anemia [10–12], and chronic erythremic myelosis [13] The anemias are frequently megaloblastic [6–8 14] Hemochromatosis has also been documented in hereditary hypochromic anemias [15 16] Secondary hemochromatosis must be extremely rare in patients with aplastic anemia who do not have an erythroplastic marrow [17] Patients who develop hemochromatosis in association with anemia have frequently received large amounts of transfused blood It was once thought that the transfused red cells represented the sole source of the increased iron stores found at autopsy Houston however reported a case in a man with long standing macrocytic refractory anemia who had been given no transfusions [18] Secondary hemochromatosis has been reported in patients with mild anemia who have received no transfusions but who have received large doses of medicinal iron for many years [ 19] One female patient was presumed to have taken over 25 000 gm iron over a 47 year period [20]

Secondary hemochromatosis is frequently indistinguishable pathologically from the idiopathic type [9 12 15 18] The pathologic changes usually develop while the patient has a normal dietary iron intake Increased iron absorption has also been demonstrated in this group of patients [21] The secondary type differs from the primary in that it may begin in childhood [22] and there is equal sex incidence

*Siderosis* is a condition characterized by increased tissue iron without many of the clinical and pathologic features of either primary or secondary hemochromatosis The term was first used by Quincke [23] for accumulated iron deposits in the tissues and organs under various conditions Localized accumulations of iron may occur as a result of hemorrhage into the tissues producing a siderosis localized to a particular tissue as in pulmonary hemosiderosis Neumann [24] first proposed the term *hemosiderin* for the microscopically visible tissue iron containing brown pigment

*Transfusion hemosiderosis* is due entirely to the overloading of the tissues from iron contained in transfused red cells and is thus a true hemosiderosis since the excess stores arise from hemoglobin iron The first record of an example of this condition was made by Kark who described two cases of aplastic anemia [25] Since that time the condition though rare has been reported in about seventy cases [26–33] The quantities of iron deposited in the tissues usually do not equal those found in hemochromatosis except when 200 or more blood transfusions have been given The iron is deposited predominantly in the reticuloendothelial rather than the parenchymal cells and is therefore especially heavy in the bone marrow kidney and spleen [33 34] The serum iron may be normal Finely granular cirrhosis of the liver cardiac dysfunction and fibrosis of the pancreas are not found diabetes mellitus gonadal hypoplasia and skin pigmentation occur infrequently and are usually mild Increased iron absorption probably does not occur in patients not having erythroid hyperplasia but patients with this latter condition may have increased tissue iron stores secondary to transfused red cell iron and increased iron absorption

*Cytosiderosis* is endemic in the Bantu in certain areas in South Africa and is probably not a specific disease entity It is a pathologic process that appears to begin in childhood [35 36] and occurs in individuals with malnutrition or pellagra whose diet contains very large amounts of iron [37] It may progress to fibrosis and siderosis of the liver but diabetes and fibrosis of the pancreas do not occur and cardiac function is not impaired [38] Increased tissue iron may also occur in severe malnutrition [39]

*Kaschin Beck disease* is a form of iron storage disease that appears to be restricted largely to Manchurian hunters and farmers [40] and is endemic in areas where drinking water with a very high iron content (0 3 to 1 0 mg per liter) is consumed during childhood and puberty The

major changes appear in the skeletal system as shortened long bones and short stature joint deformities and arthritis particularly of the inter phalangeal joints A minimal fibrosis of the liver may occur without cirrhosis pancreatic fibrosis, or cardiac dysfunction Skin pigmentation and elevated serum iron values may be present

## CLINICAL FEATURES

Hemochromatosis occurs sporadically throughout the world and although it is rare cases have been reported from most countries There appears to be no particular geographic distribution but the incidence may be lower in areas where iron deficiency is prevalent and higher in areas where increased dietary iron intake or certain refractory anemias are more common [41 42] Environmental and dietary factors other than iron intake may play a role in influencing the age of onset of the disease The frequency of the disease has not been correlated with occupation or exposure to toxic agents

Extensive descriptions of the clinical features of hemochromatosis have been presented by Sheldon [41] Heilmeyer [43] and several American authors [42, 44–46]

### *Symptoms and Signs*

The asymptomatic interval preceding the development of the clinical features covers many years and the disease is seldom recognized during this stage unless by chance as the result of a tissue biopsy or plasma iron determination Although symptoms and signs may have their onset earlier the full complex has not been reported prior to the age of 20 years [41] Hemochromatosis developing in early adulthood is frequently fulminating and carries an unfavorable prognosis In most cases the symptoms start in the middle years (in 90 per cent after the age of 30) The disease starts earlier in males and is ten to twenty times more common in males than in females The comparative rarity of hemochromatosis in females and the later age of onset may be due to the increased iron deficits that result from menstruation pregnancy and lactation Throughout the sexually productive years the average woman may lose from 10 to 15 gm iron through menstruation A single pregnancy and lactation may contribute to the loss of another 0 5 gm iron

The symptoms most frequently encountered initially are those related to the classical triad of diabetes skin pigmentation and cirrhosis Although the liver is the first tissue to contain large deposits of iron the early symptoms of weakness malaise and weight loss may most likely be related to the diabetes mellitus Skin pigmentation develops insidiously and is followed by symptoms related to cirrhosis of the liver e g abdominal pain and ascites Dyspnea and edema resulting from impaired cardiac

function and loss of libido as a consequence of involvement of the endocrine organs (testes) may next make their appearance

Skin pigmentation and hepatomegaly are the most constant *physical signs* of hemochromatosis and develop earliest The brownish pigmentation is primarily the result of increased melanin deposition in the skin and appears early, with the increased deposition of iron in the integument a brownish gray (metallic bronze or slate) color makes its appearance Pigmentation of the skin is usually generalized but it appears first and is most intense over the exposed areas and extensor surfaces of the arms external genitalia nipples and scars Except for the less frequent mucous membrane pigmentation the distribution and appearance of the brownish pigmentation resembles that seen in Addison s disease [47]

The liver usually becomes enlarged long before signs and symptoms of hepatic dysfunction develop Fibrosis and dysfunction of the liver underlie such characteristic signs of cirrhosis as spider angiomas and palmar erythema Body depilation loss of libido testicular atrophy and gynecomastia may also be partially related to liver failure With the steady progression of fibrosis and liver failure, the signs and symptoms and liver function test results become indistinguishable from those of typical Laennec s portal cirrhosis With the onset of portal hypertension splenomegaly may appear Such complications as ascites edema and bleeding from esophageal varices appear to be much less common in hemochromatosis with cirrhosis than in typical Laennec s cirrhosis Many patients with hemochromatosis of long duration develop primary carcinoma of the liver [42] Cardiac failure and primary carcinoma of the liver may frequently contribute to the ascites and edema

X ray examination of the abdomen may demonstrate an increased density of the liver because of the high iron content This is sometimes most prominent as a sharp line just below the shadow of the right diaphragm [42]

The myocardium is gradually involved this is manifested by the signs and symptoms of congestive failure and cardiac arrhythmias [48 49] When cardiac failure develops there usually is dilatation of both ventricles with a globular cardiac profile resembling that occurring with pericardial effusion In the younger subjects cardiac disturbances may progress rapidly and lead to sudden death Cardiac failure now constitutes the single leading cause of death in hemochromatosis prior to the use of insulin the leading cause was diabetes Hypertension is rarely seen in hemochromatosis

Endocrine dysfunction is manifested by loss of body hair testicular atrophy gynecomastia and menstrual irregularities Loss of libido progressing to complete impotence may develop The e signs may in part be the result of impaired liver function

Pain in the extremities cutaneous hyperesthesia and muscle and leg

cramps frequently appear as a late complication of hemochromatosis these symptoms do not seem to be attributable to the diabetes mellitus Psychic disturbances may also appear, and iron may be deposited in various areas of the brain [50]

Iron deposits occur focally in the renal tubules but do not affect renal function There is no significant hematologic disturbance in primary hemochromatosis and the bone marrow is usually normal The absence of hematologic involvement usually differentiates the idiopathic or primary disease from secondary hemochromatosis

## PATHOLOGY

The pathology of hemochromatosis has been frequently reviewed [28-32 41 42 51]

The liver is usually greatly enlarged and has a rusty appearance with a ' hobnail surface Microscopically, there is an advanced finely granular cirrhosis of the multilobular or portal type Areas of regeneration are prominent There is degeneration of the liver cells and a large amount of the iron may be present extracellularly or within the Kupffer cells The large size of the liver the small regenerative nodules of liver cells and the absence of broad bands of atrophy characteristically differentiate this disease from postnecrotic cirrhosis Hemosiderin and hemofuscin pigments are present and the former is strikingly increased within the liver parenchymal cells In the various other forms of siderosis the iron content of the liver is usually not so great and the largest quantity of iron is within the Kupffer rather than the parenchymal cells [30 33, 36] In typical Laennec s cirrhosis with siderosis the iron is also predominantly within the Kupffer cells and the liver is usually small [31] Although fibrosis of the liver may occur in other forms of siderosis finely granular cirrhosis does not [30 32 36]

The pancreas shows a heavy brown discoloration and except for the liver contains the largest amount of iron There is a great increase in fibrous tissue The gland cells show degenerative changes and siderosis and in most cases the islet cells are affected It has been proposed on the basis of studies of 62 patients with diabetes secondary to hemochromatosis that the diabetes does not predispose to renovascular disease and that the diabetes of hemochromatosis probably represents a true insulin deficiency [52] Diabetes mellitus occurs less frequently in siderosis uncomplicated by secondary hemochromatosis [9 33] The salivary glands like the pancreas usually show a deep brown red discoloration and the glandular epithelium contains large deposits of hemosiderin

The spleen is usually only slightly enlarged Hemosiderin deposits appear in very much smaller amounts than in the liver and pancreas This

is in contrast to the greater splenomegaly and much higher iron content of the bone marrow kidney and spleen in transfusion hemosiderosis [33 34]

The mucous membrane of the entire gastrointestinal tract may be pigmented The hemosiderin deposits are heaviest in the glandular epithelium of the stomach duodenum and cecum although there appears to be no alteration in the structure of the organs

The heart is usually a deep brown color Microscopically the endocardium and pericardium are not involved and the pathologic changes are confined entirely to the myocardium which may contain large amounts of hemosiderin Degenerative changes are seen in the muscle fibers and the increased fibrous tissue may replace the muscle

The pituitary usually shows a brown discoloration from increased deposits of hemosiderin localized to the cells of the anterior lobe The *testes are usually atrophic and on microscopic examination show* atrophy of the germinal epithelium The deposits of hemosiderin are usually not large and fibrous tissue is not increased The ovaries usually do not contain increased quantities of hemosiderin The parathyroid glands are frequently heavily pigmented without overgrowth of fibrous tissue The thyroid also shows a brownish pigmentation and microscopically there are marked overgrowth of fibrous tissue and increased quantities of hemosiderin within the alveolar epithelium

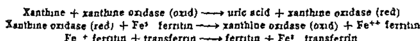
The adrenal glands do not appear altered grossly and there is no increase in connective tissue The microscopic changes are localized to the cortex and in most cases are confined to the outer zona glomerulosa Hemosiderin when present is always found in the glomerulosa and frequently only in this zone Hellier [47] reported a correlation between the degree of melanin pigmentation and the amount of iron deposited in the zona glomerulosa At autopsy one case of classical hemochromatosis with minimal pigmentation was found to have no iron deposits in the zona glomerulosa [53] In the various forms of siderosis the endocrine organs are not usually heavily laden with iron except for the deposits within the zona glomerulosa Some investigators [47 53–55] have alluded to a possible relationship between the skin pigmentation infrequent hypertension and more frequent hypotension and adrenal insufficiency secondary to the iron deposits in the adrenal cortex Studies of adrenal function in hemochromatosis have not been made although low values for both urine corticosteroid and 17 ketosteroid might be expected because of the cirrhosis [56] Simpson [57] reviewed the reported cases of Addison s disease with diabetes mellitus and could find only two with hemochromatosis Since the zona glomerulosa is most heavily laden with iron deposits it might be expected that aldo terone metabolism would be altered earliest

the apoferritin content of the mucosal cells of the stomach and duodenum [79] Ferritin is not normally present in the plasma but it may appear there in patients with toxic hepatitis [80]

Hemosiderin is the iron hydroxide–protein complex that is visible microscopically in the tissues as brownish granules It remains insoluble under the conditions (neutral aqueous solution) used for the extraction of ferritin from the tissues [81] Hemosiderin contains relatively less protein than ferritin and consequently more iron The iron content may range from 30 to 35 per cent and the iron to nitrogen ratio is 5–9 1 Hemosiderin and ferritin both give the Prussian blue reaction but in tissues the visible Prussian blue color is usually produced only by hemosiderin that is clumped in large granules Studies with the electron microscope show that some of the hemosiderin deposits are contained within membranes ('siderosomes ) and that some of the brown clusters of pigment may be predominantly ferritin [82] Thus a significant portion of apparent hemosiderin may represent iron rich molecules of ferritin Both hemosiderin and ferritin iron are utilized for hematopoiesis [83 84] although the latter is probably utilized more efficiently

Transport iron in the plasma (3 to 4 mg) represents the link between the various tissue iron compartments In the presence of oxygen ferrous iron entering the plasma is quickly oxidized to the ferric form which is then transported by a specific iron binding $\beta$ globulin siderophyllin or transferrin [85] The trivalent iron is bound to this protein with ionic bonds [86] Normally the iron binding protein accounts for only about 3 per cent of the total plasma protein and is only about one third saturated with iron Transferrin in man is not a single protein species The different transferrins have been identified as the proteins involved in the genetically controlled variations of the serum $\beta$ globulins in human beings [87] A deficiency of transferrin has not been found in any disease This protein has a molecular weight of 90 000 and can chelate a maximum of two iron atoms in the trivalent state (five-odd electrons) The transferrin forms a strong ionic bond with iron in neutral or alkaline solution to give a salmon pink color with an absorption maximum at 470 m$\mu$ This property has made possible methods for its assay [88] In vitro the reaction between iron and transferrin is reversible and is dependent on pH The affinity constant at pH 7 is $10^7$ At physiologic pH the ionic iron concentration in the plasma is related to the level of bound Fe-transferrin and the concentration of unbound transferrin Because of the high molecular weight of the Fe transferrin it is unlikely that the complex normally leaves the vascular bed in more than trace amounts and thus the iron is presumed to pass in and out of the blood stream in ionized form [89] e g after oral iron feeding the plasma iron level increases without a change in the transferrin content Normally transferrin has a biologic half life of approximately 12 days [90]

The biochemical mechanism for the release of iron from the tissue ferritin to the plasma transferrin has recently been investigated by Mazur and his coworkers In rat liver slices in vitro [91] and in rabbits guinea pigs, and dogs in vivo [92] the enzyme xanthine oxidase was shown to participate in the release of iron from liver ferritin Tissue hypoxia was induced in all these experiments and was associated with an increase in purines in the liver tissue Under anaerobic conditions and in the presence of ferritin the purines xanthine and hypoxanthine were oxidized to uric acid by the dehydrogenase activity of xanthine oxidase In this reaction ferritin acts as an electron acceptor and its iron is reduced to the ferrous state The ferrous iron can then be removed from the protein moiety by the plasma transferrin


Xanthine + xanthine oxidase (oxid) ⟶ uric acid + xanthine oxidase (red)
Xanthine oxidase (red) + $Fe^{+++}$ ferritin ⟶ xanthine oxidase (oxid) + $Fe^{++}$ ferritin
$Fe^{++}$ ferritin + transferrin ⟶ ferritin + $Fe^{+++}$ transferrin

Thus the tissue oxygen tension levels of xanthine oxidase and xanthine oxidase substrates all may influence the regulation of the iron content of the circulating plasma

Little is known of the mechanism for release of iron from the plasma transferrin for cellular utilization On the basis of recent studies with the electron microscope it has been suggested that the immature red cells (erythroblasts) utilize a process called pinocytosis to obtain iron for hemoglobin synthesis by encompassing ferritin molecules directly from macrophages lying adjacent to the erythroblasts [93] The importance of such a mechanism for incorporation of iron into marrow erythroblasts has not been established It is known from in vitro studies that immature red cells are capable of taking up iron bound to the plasma protein transferrin [94 95] Also there appears to be a very efficient system in the placenta for the transfer of plasma protein bound iron to the fetal circulation [96] The reaction in vivo between ferric iron and protoporphyrin to form heme appears to be enzyme catalyzed [97] In rat liver the enzyme is found in the mitochondria [98]

*Iron Excretion*

The high molecular weight of transferrin and its great affinity for iron probably serve to protect iron from excretion Normally less than 1 mg is excreted per day in the urine and feces [99] As the result of menstrual loss the female may lose an average of about 1 mg additional iron per day The concentration of iron in the bile is 1 to 2 mg per liter however most of this is reabsorbed so that normally less than 0 5 mg is excreted each day [100] The urine normally contains less than 0 5 mg iron per 24 hr [101] In the presence of increased iron stores in man [102] and animals [103] iron excretion is usually slightly increased It is decreased in states

of iron deficiency In nephrosis large quantities of the iron transferrin complex may be excreted [104] and the urine may contain more than 1 5 mg iron per day [105]

*Iron Absorption*

The normal adult accumulates his total body iron slowly during the first 20 years of life and thereafter remains in iron balance The amount *gaining access to the body by absorption* equals the amount *lost by* excretion (0 5 to 1 5 mg per day) Alterations in excretion are minimal and it is generally agreed that the body content of iron is largely dependent on the amount absorbed McCance and Widdowson [106] on the basis of their observations on the minimal excretion of iron from the body suggested that *the mucosal cells of the gastrointestinal tract have an* active role in the control of iron absorption Hahn [107] from studies with radioactive iron found that iron absorption is not increased until 6 to 7 days after an acute hemorrhage he suggested that the gastrointestinal mucosa does not increase iron absorption until the body iron stores have been depleted as a result of the accelerated hematopoiesis He and others [108] found that the antecedent oral feeding of a large quantity of stable iron would block or inhibit the absorption of a dose of radioactive iron administered subsequently and proposed a 'mucosal block' to explain this observation Granick [79] observed that feeding increasing amounts of iron increases the synthesis of apoferritin and results in the formation of increased amounts of ferritin in the intestinal mucosa According to Granick when only minimal amounts of iron are made available from the lumen of the gastrointestinal tract iron reaches the blood directly through the mucosa However if much ferritin is present in the mucosa it prevents further iron uptake It was therefore postulated that the amount of iron absorbed depends on *the relative* state of saturation of the mucosal cells with iron and in turn is related to the level of tissue iron stores Heilmeyer [109] and Wohler [110] failed to find evidence that the increased ferritin content of the intestinal mucosa reduces iron absorption When the ferritin content of the mucosal *epithelium was maximal iron absorption continued and* hemosiderin continued to increase in the interstitial tissues of the mucosa If iron is given in excessive quantities [111] the mucosal cells of the intestine are unable to prevent its accumulation in excess of body needs When more iron is present in the gut more is absorbed although the fraction absorbed may be smaller [112 113]

The concept of the mucosal block fails to explain the excessive absorption and heavy tissue iron deposits found in some subjects after the prolonged ingestion of large amounts of dietary [37 38] or medicinal iron [5 20] Moreover the diets of patients with primary and secondary hemochromatosis may contain a normal amount of iron yet its absorp-

tion may be greater than normal [*7, 8, 15 18 114–117*] Also following experimentally induced hypochromic anemia in animals [*118*] and in normal subjects [*63 64*] iron absorption is increased After the anemia is corrected even though the iron stores remain depleted iron absorption reverts to normal In animals with experimentally induced anemia and increased iron stores iron absorption remains elevated [*119 120*] though it may be slightly depressed in animals when tissue iron stores are increased without an associated anemia [*103 118 121*] In the e circum stances the ferritin content of the duodenum is elevated but the excess ferritin is deposited in the submucosa rather than in the mucosal cells [*110*]

The normal dietary intake of iron is 10 to 15 mg Less than 10 per cent of this quantity is absorbed through the gastrointestinal mucosa The main sites for absorption of iron appear to be the stomach and duodenum Other areas of the intestine absorb iron if it is present in a soluble form [*122 123*] Once absorbed the iron is transported via the portal vein *blood to the liver In animals it has been shown that the intestinal* lymphatic vessels do not transport the absorbed iron [*124 125*]

Both local intraluminal factors within the gastrointestinal tract and systemic or internal factors may influence iron absorption [*126*] With the use of $Fe^{59}$ salts it has been demonstrated that the normal adult absorbs 1 to 10 per cent of an oral dose of ferric iron salts if the dose is in the microgram to milligram range [*127*] With larger than milligram quantities the percentage absorbed decreases in proportion to the amount administered From 5 to 20 per cent of administered ferrous iron is absorbed Usually less than 10 per cent of food iron is absorbed [*117 128 129*] and the fraction absorbed varies with different foods Administration of a reducing agent such as ascorbic acid promotes the absorption of ferric salts but does not greatly promote absorption of food iron It has been shown experimentally that a low gastric pH will increase iron absorption by promoting the reduction of ferric iron and increasing the solubility of the iron salts Gastric acidity does not appear however to be necessary for absorption of the normal daily iron requirements Subjects with gastric achlorhydria do not show decreased absorption of iron and the addition of HCl to the feeding does not result in an increased absorp tion [*128*] The presence of phosphates or other substances capable of forming insoluble ferric or ferrous salts reduces the availability of iron for absorption [*127 129 130*] The presence of accessory factors that form soluble iron complexes (citrate) increases iron absorption Local secretions within the gastrointestinal tract may influence iron absorption e g ligation of the pancreatic ducts increases iron absorption in cats [*131*] and ethionine induced pancreatic damage is associated with a similar result in rats and mice [*132*]

The rate of erythropoiesis probably is the most important internal

factor influencing iron absorption [21, 113, 117] Patients with megaloblastic hemolytic and chronic iron deficiency anemias absorb increased quantities of iron, although in the first two groups iron stores are increased Iron absorption is increased in experimentally induced phenylhydrazine anemia in dogs [119] and rats [120] Following acute blood loss increased absorption is not noted for 5 to 7 days which is the time necessary for development of marrow erythroid hyperplasia [107, 113] In man altitude polycythemia will temporarily increase iron absorption [133] and hypoxia produces the same effect in rats [134] These physiologic states of lowered oxygen tension are associated with erythroid hyperplasia Patients with aplastic aregenerative anemias and marrow hypoplasia do not absorb increased amounts of iron, and do not develop siderosis unless they are transfused with red cells Transfusions of whole blood in sufficient quantities to depress marrow activity decrease iron absorption [113] In the absence of erythroid hyperplasia anemia per se and hypoxia do not increase iron absorption The anemia of infection is associated with decreased iron absorption at a time when the tissue iron may be normal or slightly increased [135] Pyridoxine deficiency in swine which is characterized by increased tissue and plasma iron levels and a hypochromic microcytic anemia with erythroid hyperplasia is associated with increased iron absorption [136] The level of the serum iron and the saturation of the plasma transferrin do not appear to influence iron absorption in dogs [137] or man [112] Decreased tissue iron stores in the absence of anemia do not promote [63 64] increased absorption of iron In rats operative removal of the major quantity of storage iron by partial hepatectomy does not lead to increased iron absorption [138] In the rat increased storage iron produces a moderate decrease in iron absorption [121, 103, 113] or no decrease if anemia is present [120]

In the normal adult in addition to increased marrow activity pregnancy also may serve as a stimulus to increased iron absorption [139, 140] Infants and growing children also absorb increased quantities of iron [141–143] It seems likely that all these stimuli to increased iron absorption are mediated through a humoral agent Although some control of iron absorption seems necessary to restrict iron accumulation in the normal nonanemic adult the iron content of the tissues of the body does not appear to be related in any clear and direct manner to the regulation of iron absorption

*Rate of Turnover of Iron*

If it is assumed that the normal adult male has a total body iron content of 4 gm and that he is in iron balance it is possible to estimate the total body iron turnover by measuring quantitatively either iron absorption or excretion If approximately 1 mg iron is absorbed and excreted each day then approximately 9 per cent of the total 4 gm body iron (0 36 gm)

would be turned over per year, and its biologic half life would be about 7 7 years Finch recently reported the results of a study on body iron turnover utilizing $Fe^{55}$ and red cell sampling over a period of 3 to 5 years [144] The calculated daily exchange of body iron (absorption or excretion) was 0 61 0 64 and 1 22 mg in men nonmenstruating women and menstruating women respectively This represented a turnover of the total body iron miscible pool (red cell iron plus miscible tissue iron) of 8 3 10 8 and 20 1 per cent per year These data demonstrate that the miscible iron pool is appreciably smaller than the total body iron content The miscible iron exclusive of the red cell iron pool probably does not include all the storage iron Total body iron turnover studies in mice [103 145] and ducks [146] have shown a more rapid turnover and the rate of turnover was proportional to body iron content

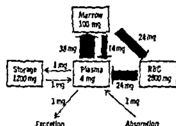


Fig 6-1 Metabolic fate of iron Normal daily rates of turnover through body compartments

After intravenous injection of $Fe^{59}$ the plasma radioactivity follows a straight line on a semilog plot for a period of 6 to 8 hr in the normal subject [147] When the curve is followed longer it deviates from linearity and may be described as a polynomial of at least two exponential terms [148–150] The rate of turnover of the plasma iron pool may be calculated as the product of the total plasma iron and the *fractional turnover rate derived from the initial slope of the curve* In the normal adult the turnover is 35 to 45 mg per day or roughly ten times the total plasma iron

Assuming a mean red cell survival of 120 days and a synthesis of 0 8 to 0 9 per cent of the red cell mass per day 20 to 25 mg of the total 35 to 45 mg of the rapidly exchangeable iron pool turned over per day is used for the synthesis of hemoglobin The 10 to 15 mg portion of the labile (marrow) iron pool and the 3 to 4 mg from extracellular fluid absorption and extramarrow stores are returned to the plasma (Fig 26-1) This turnover *may be increased in hemochromatosis more* because of an increase in the total plasma iron than because of an increase in the fractional turnover rate

The complexity of the *$Fe^{59}$ decay curve in plasma indicates that the* plasma iron is miscible with several other compartments Some of these are indicated in Fig 26-1 From the $Fe^{59}$ data it is possible to calculate the iron content of the labile iron pool the quantity of iron that daily re enters the plasma from this labile pool and the amount of iron leaving the labile pool each day for hemoglobin synthesis From a knowledge of the iron content of hemoglobin it is possible to calculate the rate of hemoglobin synthesis

## IRON METABOLISM IN HEMOCHROMATOSIS

In primary hemochromatosis, the quantity of iron in the diet is presumed to be normal. It would seem then that any explanation of the increased iron stores must assume an increased iron absorption at some stage of development of the disease. Quantitative measurements of iron absorption in hemochromatosis have yielded variable results [*114–117, 120, 139, 151, 152*]. This is probably partially attributable to the fact that iron balance studies utilizing radioactive iron yield variable results in normal subjects and because no strongly positive iron balance is needed to produce the increased iron stores so typical of this disease. The average patient with hemochromatosis has an excess store of 20 gm or more of iron at the time of diagnosis (35 or more years of age). This represents a positive balance of 2 to 4 mg per day over a period of 20 to 30 years or on a diet containing 10 to 20 mg iron per day an absorption of 20 to 30 per cent of the dietary iron rather than the normal 5 to 10 per cent. In a few patients the dietary history points to an increased iron intake secondary to the indiscriminate use of medicinal preparations of iron. These cases are more correctly classified as secondary hemochromatosis.

In a few studies of younger patients with hemochromatosis $Fe^{59}$ balance techniques have demonstrated increased iron absorption [*114–117, 151, 152*]. In a recent study [*117*] in 5 patients an average of 10 per cent of an orally administered dose of radioiron added to a standard test meal was absorbed in contrast to an average of 5 per cent in normal persons. After phlebotomy the disparity between these two groups become more marked and up to 30 per cent absorption was found in patients with hemochromatosis at a time when the serum iron and iron stores were still high. This degree of absorption is more than is seen in patients with iron deficiency anemia or in normal subjects after phlebotomies.

Removal of injected $Fe^{59}$ from the plasma of patients with hemochromatosis may be slow. Also the plasma $Fe^{59}$ disappearance curve may show three distinct first-order processes rather than the two usually seen in normal subjects [*150*]. This suggests that significant amounts of the $Fe^{59}$ reenter the plasma pool from at least two labile iron pool. Measurements of the slopes of the zero time intercepts permit a determination of the total quantity of iron removed via the marrow and storage each day, the quantity of iron in the labile marrow pool and other storage (liver) pool, and the turnover of iron from the labile pool of the marrow used for hemoglobin synthesis. The plasma iron turnover is frequently increased [*150, 152, 153*] but not the rate of red cell synthesis. Red cell life span is normal. Since there is no increased synthesis of red cells in hemochromatosis, increased erythroid activity, a known stimulus to

increased iron absorption would not seem to be an etiologic factor in the pathogenesis of the disease

After the intravenous injection of $Fe^{59}$ in vivo measurements over the liver show initially a greater than normal accumulation of iron in the liver This continues for several days and demonstrates the great capacity of the liver to continue to accumulate iron in the presence of excess iron stores [154]

*Iron Overload Studies in Animals*

Accumulations of iron have been produced in the tissues of experi mental animals by various means In no case has diabetes cirrhosis or myocardial damage followed [121 155–162] Accordingly there has been a reluctance to attribute the tissue damage in hemochromatosis to iron However experimental animals have usually not been fed dietary iron over a sufficiently long period of time to permit the accumulation of iron in amounts comparable to that seen in hemochromatosis Furthermore injected iron is distributed primarily in the reticuloendothelial system rather than in the parenchymal cells as in hemochromatosis In both hemochromatosis and transfusion hemosiderosis (with secondary hemo chromatosis) it seems necessary for the body iron content to reach 20 to 30 gm before the clinical picture of classical hemochromatosis makes its appearance In man if the bulk of the tissue iron is derived from trans fused red cells more than 50 gm iron may be present without any signs of severe tissue injury such as cirrhosis [163] In the dog after the ad ministration of 25 to 35 gm iron intravenously death results within 5 to 10 months but no fibrous tissue reaction and no diabetes or cirrhosis is found [164]

## TREATMENT

The treatment of hemochromatosis should be directed at mobilization and removal of the excess tissue iron Repeated phlebotomies are known to be effective in accomplishing these aims [63–65 114 165–178] Patients with hemochromatosis withstand frequent phlebotomies (500 ml or more of whole blood each 4 to 6 days for many months) without developing more than a mild anemia This implies that with this bleeding regimen the patients are able to increase their blood production four to sixfold above the normal rate Failure to maintain a hematocrit above 35 with weekly phlebotomies suggests the presence of a complicating disease such as hepatoma or infection Even in patients with a moderate degree of cirrhosis it is usually not necessary to reinfuse the plasma

In order to have an index of the extent of the iron stores it is desirable to do a liver biopsy before starting the series of phlebotomies Since each 1 ml of packed red cells contains approximately 1 mg iron it is possible to remove only 10 to 15 gm iron per year and thus in an average patient

the phlebotomies may have to be continued for 2 to 3 years A gradual fall in the serum iron is usually the first sign of an approaching depletion of the iron stores when this occurs the interval between phlebotomies may be lengthened A gradual decline in the hematocrit follows indicating a depletion of the iron stores A repeat liver biopsy serves to confirm the presence of depleted iron stores

It does not seem necessary to restrict the normal dietary intake of iron or to add phosphates to the diet since the amount of iron absorbed during the period of phlebotomies is small compared with that lost through the bleedings Because these patients probably absorb an increased amount of the ingested food iron, it may be necessary to continue phlebotomies indefinitely at intervals of 3 to 6 weeks The chelating agents presently available have no place in the therapy of hemochromatosis because they remove only trace quantities of tissue iron via the urine [*174 175*]

*Tissue injury (fibrosis of the liver and pancreas) and the clinical mani*festations of hepatic, cardiac and pancreatic islet cell insufficiency skin pigmentation and endocrine disturbances in patients with primary or secondary hemochromatosis strongly suggest a relationship between the iron content of the tissues and their injury Reversal of many of the symptoms and signs and the lack of progression of the clinical picture of hemochromatosis in patients treated by phlebotomy also suggest that the pathologic features of hemochromatosis may be related to excess tissue iron Since phlebotomy therapy has been in use for less than 10 years it is too early to predict whether it will materially alter the prognosis However experience to date demonstrates that the skin pigmentation decreases cardiac function improves liver dysfunction does not progress and the diabetes may be improved as judged by a reduction in insulin requirement This form of therapy may not alleviate all the manifestations of the disease but it may well prevent further irreversible tissue damage

## GENETICS

Hemochromatosis occurs in both adult males and females although it usually occurs earlier in males Thus it is not transmitted as a sex linked defect The disease appears sporadically Following the initial case report of Frisch [*176*] on its occurrence in a family numerous reports of familial cases appeared in the literature Sheldon [*41*] evaluated the reported familial incidence in 14 cases but the data appeared reliable in only 5 of the families Boulin [*55*] reviewed the data from a total of 27 cases with familial histories reported in 1953 The disease has been reported in 2 or more brothers in more than 25 different families [*42 176–190*] It has been observed in identical twin brothers on three occasions [*180 182, 183*] There are five reports of the disease occurring in successive

generations [*42 182 185 186 191*] There is one report of the disease occurring in a mother and her identical twin sons [*182*] and another report of the disea e in both the mother and father, and a daughter [*185*] There are three case reports of the disease occurring as the result of consanguineous matings [*186 190 191*] In one such family appearance of a severe form of the disease in early adult life in three sisters and a brother [*190*] suggests the presence of a more severe juvenile form of the disease

The frequent occurrence of an elevated serum iron has been noted in the siblings or children of patients with hemochromatosis [*42 186 192*] In the study of Debré et al [*186*] elevated levels were not present in male or female offspring under age 15 nor in female offspring over 15 In males over 15 almost half showed elevated serum iron levels Also hyperpigmentation of the skin and diabetes have frequently been reported in relatives (children parents siblings) of patients with hemochromatosis Increased levels of serum iron and increased iron stores as measured by phlebotomy have been reported in the siblings of patients [*42*]

An analogy between elevated serum iron levels and hemochromatosis on the one hand and hyperuricemia and clinical gout on the other is perhaps instructive In both disorders the elevated serum concentration of the characteristic chemical substance is encountered much more frequently than the associated disea_e The on et of the chemical defect at puberty in males has been noted in both disorders Careful studies of inheritance of hyperuricemia have shown that it behaves as a dominant factor when viewed in successive generations but as a polymeric factor when viewed in sibling groups (Chap 21) One could equally well postulate that the genetic factors determining serum iron levels and hemochromatosis are polymeric On this basis one would expect elevated serum iron levels and hemochromatosis to occur in successive generations and in siblings and one would expect a severe and perhaps juvenile form of the disease in individuals inheriting it from both parents as in offspring of consanguineous matings

Certain of the family data are also compatible with inheritance as an autosomal dominant trait with incomplete penetrance or even as an autosomal recessive characteristic However it appears unlikely to the author that the quantitative aspects of so complex a process as iron absorption would be determined by a single autosomal gene It is not known whether the genetically determined defect leading to increa ed iron absorption is in the mucosal cells or in some other body cellular enzyme system which in turn influences the absorptive capacity of the muco al cells

I urther studies of genetic factors in hemochromatosis are needed The combined application of studies of iron absorption and of serum iron

levels in families of patients with hemochromatosis offers a promising outlook for the acquisition of important information

## SUMMARY

1 Primary hemochromatosis is a form of iron storage disease characterized by excessive deposits of an iron hydroxide–protein complex called *hemosiderin* in various organs of the body It is one of several forms of iron storage disease, and is not associated with known causes of excessive iron absorption such as erythroid hyperplasia

2 Deposits of hemosiderin occur in liver heart, the endocrine organs and elsewhere The signs and symptoms of the disease arise from damage done these organs by the tissue reaction to the hemosiderin deposits There is a curious metallic slate blue to brown pigmentation of the skin which is primarily due to melanin but also to deposition of hemosiderin The pigmentation hepatic cirrhosis and diabetes of the insulin lack variety form the classical triad of the disease

3 There is a small daily excessive absorption of iron in primary hemochromatosis The cause for this is entirely unknown at present There is no excessive excretion of iron The net positive balance is only 2 to 4 mg per day This excessive iron absorption is not associated with the common cause of increased iron absorption which is increased erythropoiesis

4 There is no increase in intestinal ferritin in primary hemochromatosis nor is there any increase in plasma transferrin, the $\beta$ globulin protein which normally transports iron The plasma transferrin is more nearly saturated with $Fe^{3+}$ than normally The turnover of plasma iron is increased

5 Treatment of the disease if begun early is helpful It consists of systematic depletion of the iron stores by phlebotomy at appropriate intervals

6 The disease probably has an autosomal recessive pattern of inheritance which is partially sex limited by virtue of the periodic loss of iron accompanying menstruation Some families are reported in which the inheritance is more in keeping with a dominant form with incomplete penetrance Hyperpigmentation of the skin increased concentrations of serum iron and increased intestinal absorption of iron have been reported in otherwise normal relatives of patient with hemochromatosis indicating partial expression in the heterozygous state

## BIBLIOGRAPHY

1 Troisier M Diabète sucré Bull soc anat Paris 16 231 1871
2 Trousseau A *Clinique médicale de l Hotel Dieu de Paris* 2d ed p 67 Baillière Paris 1865
3 von Recklinghausen F D Über Hamochromatose Tagebl der Versamml Natur u Ärzte Heidelberg 62 324 1889

4 Sheldon J H The iron content of the tissues in hemochromatosis with special reference to the brain Quart J Med 21 123 1927
5 Wallerstein R O and Robbins S L Hemochromatosis after prolonged iron therapy in a patient with chronic hemolytic anemia Am J Med 14 256 1953
6 Kozewski B J Occurrence of megaloblastic erythropoiesis in patients with hemochromatosis Blood 7 1182 1952
7 Granville N and Dameshek W Hemochromatosis with megaloblastic anemia responding to folic acid report of a case New England J Med 258 586 1958
8 Maier C Megaloblastare Vitamin $B_6$-Mangelanamie bei Hamochromatose Schweiz med Wchnschr 87 1234 1957
9 Block M Hemosiderosis and hemochromatosis in *Iron in Clinical Medicine* edited by R O Wallerstein and S R Mettier p 115 University of California Press Berkeley Calif 1958
10 Ellis J T Schulman I and Smith C F Generalized siderosis with fibrosis of liver and pancreas in Cooley's (Mediterranean) anemia With observations on the pathogenesis of siderosis and fibrosis Am J Path 30 287 1954
11 Frumin A M Waldman S and Morris I Exogenous hemochromatosis in Mediterranean anemia Pediatrics 9 290 1952
12 Howell J and Wyatt J P Development of pigmentary cirrhosis in Cooley's anemia Arch Path 55 423 1953
13 Dickerman R C Rom J and Kobernick S D Chronic erythremic myelosis with exogenous hemochromatosis Am J Clin Path 27 56 1957
14 Heffernan C K and Jaswon N A case of paroxysmal nocturnal haemoglobinuria associated with secondary haemochromatosis a lower nephron nephrosis and a megaloblastic anaemia J Clin Path 8 211 1955
15 Goldish R J and Aufderheide A C Secondary hemochromatosis II Report of a case not attributable to blood transfusion Blood 8 837 1953
16 Caroli J Bessis M Combrisson A Malassenet R and Breton J Hémochromatose avec anémie hypochrome et absence d hémoglobine anormal Presse méd 65 1991 1957
17 Morningstar W A Exogenous hemochromatosis Arch Path 59 355 1955
18 Houston J C Hemochromatosis and refractory anemia Guy s Hosp Rep 100 355 1951
19 Case Records of the Massachusetts General Hospital New England J Med 247 99 1952
20 Case Records of the Massachusetts General Hospital New England J Med 258 692 1958
21 Dubach R Callender S T and Moore C V Studies in iron transportation and metabolism absorption of radioactive iron in patients with fever and with anemias of varied etiology Blood 3 526 1948
22 Chesner C Hemochromatosis J Lab & Clin Med 31 1029 1946
23 Quincke H Zur Pathologie des Blutes Siderose Arch path Anat 95 125 1880
24 Neumann E Beitrage zur Kenntnis der pathologischen Pigmente Virchow s Arch path Anat 3 25 1888
25 Kark R M Hemochromatosis One with secondary hemochromatosis following 90 transfusions in nine years and the other with secondary carcinoma of the stomach Guy s Hosp Rep 87 343 1937
26 Schwartz S O and Blumenthal S A Exogenous hemochromatosis resulting from blood transfusions Blood 3 617 1948
27 Muirhead E E Crass G Jones F and Hill J F Iron overload (hemosiderosis) aggravated by blood transfusions Arch Int Med 83 477 1949
28 Aufderheide A C Horns H L and Goldish R J Secondary hemochromatosis I Transfusion (exogenous) hemochromatosis Blood 8 824 1953
29 Kleckner M S Baggenstoss A H and Weir J F Hemochromatosis and transfusional hemosiderosis a clinical and pathologic study Am J Med 16 382 1954
30 Kleckner M S Baggenstoss A A and Weir J A Iron storage diseases Am J Clin Path 25 915 1955

31 Dubin I N Idiopathic hemochromatosis and transfusion siderosis Am J Clin Path 25 514 1955
32 Wyatt J P Patterns of pathological iron storage A M A Arch Path 61 49 1956
33 Oliver R A M Siderosis following transfusions of blood J Path & Bact., 77 171 1959
34 Schwartz S Exogenous hemochromatosis Concept and general description Am J Clin Path 26 744 1956
35 Gillman J Mandelstam J and Gillman T A comparison of chemical and histological estimations of the iron and copper content of the livers of Africans in relation to the pathogenesis of cytosiderosis and cirrhosis (haemochromatosis) South African J M Sc 10 109 1945
36 Higginson J Gerritsen T and Walker A R P Siderosis in the Bantu of Southern Africa Am J Path 29 779 1953
37 Walker A R P and Arvidsson U B Iron intake and haemochromatosis in the Bantu Nature 166 438 1950
38 Higginson J Siderosis in Southern Africa Central African J Med 1 8 1955
39 Aoki T and Nakamura I Pathological studies on malnutrition particularly the pathology of the liver and kidney Keijo J Med 1 1 1952
40 Hiyeda K The cause of Kaschin Beck s disease Jap J Med Sc (V Pathology) 4 91 1939
41 Sheldon J H *Hemochromatosis* Oxford London 1935
42 Finch S C and Finch C A Idiopathic hemochromatosis an iron storage disease Medicine 34 381 1955
43 Heilmeyer von Ludwig Die Hämochromatose Klinik Eisenstoffwechsel und Pathogenese Acta haemat 11 137 1954
44 Butt H R and Wilder R M Hemochromatosis report of 30 cases in which diagnosis was made during life Arch Path 26 262 1938
45 Althausen T L Doig R K Weiden S Motteram R Turner C N, and Moore A Hemochromatosis investigation of 23 cases with special references to etiology nutrition iron metabolism and studies of hepatic and pancreatic function A M A Arch Int Med 88 553 1951
46 Kleckner M S Kark R M Baker L A Chapman A Z Kaplan E, and Moore T J Clinical features pathology and therapy of hemochromatosis J A M A 157 1471 1955
47 Hellier F F The nature and causation of the skin pigmentation in hemochromatosis Brit J Dermatol Syphilis 47 1 1935
48 Levin E B and Galum A. The heart in hemochromatosis Am Heart J., 45 277 1953
49 Evans J Treatment of heart failure in hemochromatosis Brit J Med 1 10 5 1959
50 Commermeyer J Deposition of iron in paraventricular areas of the human brain in hemochromatosis J Neuropath & Exper Neurol 6 111 1957
51 Hedinger C Zur Pathologie der Hamochromatose Hämochromatose als Syndrom Helvet med acta (Suppl 32) 20 1 1953
52 Lonergan P and Robbins S L Absence of intercapillary glomerulosclerosis in the diabetic patient with hemochromatosis New England J Med 260 366 1959
53 Holmes N E A case of hemochromatosis with almost complete absence of skin pigment and with fatal rupture of an esophageal varix Ann Int Med 13 1075 1939
54 Labbe M Boulin R and Ullmann Diabète bronzé et insuffisance surrénale Presse méd 44 1769 1936
55 Boulin R and Bamberger J L hémochromatose familiale Semaine hôp Paris 29 3153 1953
56 Peterson R E Adrenocortical steroid metabolism and adrenal cortical function in liver disease J Clin Invest 39 320 1960
57 Simpson S Addison s disease and diabetes mellitus in 3 patients J Clin Endocrinol 9 403 1949

58 Letulle M Not sur trois observations de cirrhose hypertrophique pigmentaire non diabétique Bull et mém m'd des hôp Paris 14 705 1897
59 Lerner A B M lanin pigmentation Am J Med 19 90 1955
60 Rath C E and Fin h C A Sternal marrow hemo iderin A method for the determination of available iron stores in man J Lab & Clin Med 33 81 1948
61 Hutchinson H E The significance of stainable iron in sternal marrow sections Blood 8 236 1953
62 Rous P Urinary siderosis haemo iderin granules in the urine as an aid in the diagnosis of pernicious anaemia haemo hromatosis and oth r diseases cau ing siderosis of the kidney J Exper Med 28 645 1918
63 Finch S Haskins D and Finch C A Iron metabolism Hematopoiesis fol lowing phlebotomy Iron as a limiting factor J Clin Invest 29 1078 1950
64 Haskins D Stevens A R Jr Finch S and Finch C A Iron metabolism Iron stores in man as measured by phlebotomy J Clin Invest 31 543 19 2
65 *Finch S C and Barnett R N Diagnostic and therapeutic phlebotomy in* hemochromatosis with anemia New England J Med 256 884 1957
66 Drabkin D L Metabolism of hemin chromoproteins Physiol Rev 31 2 6 1938
67 Demulder R Iron A M A Arch Int Med 102 254 1958
68 Granick S Ferritin its properties and significance for iron metabolism Chem Rev 38 379 1946
69 Granick S Structure and physiological function of f rritin Physiol Rev 31 489 1951
70 Farrant J L An electron microscopic study of ferritin Biochem et biophys acta 13 569 1954
71 Laufberger M Sur la cristallisation de la ferritine Bull soc chim biol 19 1573 1937
72 Theorell H Beznak M Bonnichsen R Paul K G and Akeson A On the distribution of injected radioactive iron in guinea pigs and its rate of appearance in some hemoproteins and ferritins Acta chem scandinav 5 445 1951
73 Korey S P and Levine B Effect of iron on the biosynth sis of hepatic apo ferritin J Clin Invest 34 756 1955
74 Fineberg R A and Greenberg D M Ferritin biosynthe is I Crystallization of guinea pig ferritin J Biol Chem 214 91 1955
75 Fineberg R A and Greenberg D M Ferritin biosynthesis II Acceleration of synthesis by administration of iron J Biol Chem 214 97 1955
76 Fineberg R A and Greenberg D M Ferritin biosynthesis III Apoferritin the initial product J Biol Chem 214 107 1955
77 Helwig H L and Greenberg D M Studies on the m tabolism of iron-con taining proteins with radioactive carbon and iron J Biol Chem 198 703 1952
78 Loftfield R B and Ligner E A The time required for the synthesis of a ferritin molecule in rat liver J Biol Chem 231 9 5 1958
79 Granick S Ferritin its properties and significance in gastro intestinal muco a as direct respon e to iron feeding Function of ferritin in regulation of iron absorption J Biol Chem 164 737 1946
80 Reissmann K R and Dietrich M R On the presence of ferritin in the peripheral blood of patients with hepatocellular disease J Clin Invest 35 588 1956
81 Gabrio B W Shoden A and Finch C A The relationship between ferritin and hemosiderin J Biol Chem 204 815 1953
82 Richter G W Electron micro copy of hemosiderin presence of ferritin and oc urrence of cry talline lattices in hemosiderin depo ts J Bi phy c & Biochem Cytol 4 55 1958
83 Shoden A Gabrio B W and Finch C A The relationship between ferriti n and hemosi lerin in rabbits and man J Biol Chem 204 823 1953
84 Kaldo I Stu l es on intermediary iron metaboli m VII Measurement of the iron derived from wat r soluble and water insolubl non haem compounds (ferritin and hemosiderin iron) in liver and spleen Australian J Exper Biol & M Sc 36 173 1958

85 Laurell C B Studies on the transportation and metabolism of iron in the body Acta physiol scandinav 14 Suppl 46 1947
86 Ehrenberg A and Laurell C B Magnetic measurements on crystallized Fe-transferrin isolated from the blood plasma of swine Acta chem scandinav 9 68 1955
87 Smithies O and Hiller O The genetic control of transferrins in humans Biochem J 72 121 1959
88 Rath C L and Finch C A Chemical clinical and immunological studies on the products of human plasma fractionation XXXVIII Serum Iron Transport Measurement of iron binding capacity of serum in man J Clin Invest 28 79 1949
89 Laurell C B Plasma iron and the transport of iron in the organism Pharmacol Rev 4 371 1952
90 Gitlin D Janeway C A and Farr L E Studies on the metabolism of plasma proteins in the nephrotic syndrome I Albumin α globulin and iron binding globulin J Clin Invest 35 44 1956
91 Green S and Mazur A Relation of uric acid metabolism to release of iron from hepatic ferritin J Biol Chem 227 653 1957
92 Mazur A Green S Saha A and Carleton A Mechanism of release of ferritin iron in vivo by xanthine oxidase J Clin Invest 37 1809 1958
93 Bessis M M Étude au microscope électronique de la destinée d une molécule dans l organisme la ferritine et le cycle hémoglobinique du fer Bull Acad nat méd 24 470 1958
94 Paoletti C Boiron M Tubiana M Truhart R and Bernard J Plasma iron and hemoglobin synthesis Sang 29 492 1958
95 Jandl J H Inman J K Simmons R L and Allen D W Transfer of iron from serum iron binding protein to human reticulocytes J Clin Invest 38 161 1959
96 Bothwell T H Pribilla W F Mebust W and Finch C A Iron metabolism in the pregnant rat Iron transport across the placenta Am J Physiol 193 615 1958
97 Labbe R F An enzyme which catalyzes the insertion of iron into protoporphyrin Biochim et biophys acta 31 589 1959
98 Nishida G and Labbe R F Heme biosynthesis Biochim et biophys acta 31 519 1959
99 Dubach R Moore C V and Callender S T Studies in iron transportation and metabolism IX The excretion of iron as measured by the isotope technique J Lab & Clin Med 45 599 1955
100 Ingalls R L and Johnson F A Iron from gastro intestinal sources excreted in the feces of human subjects J Nutrition 53 351 1954
101 McCance R A and Widdowson E M Iron excretion and metabolism in man Nature London 152 326 1943
102 McMahan F G A comparison of the effects of Fe-3 specific versenal and calcium disodium versenate in urinary iron excretion in a patient with hemochromatosis J Lab & Clin Med 48 589 1956
103 Chappelle E Gabrio B W Stevens A R and Finch C A Regulation of body iron content through excretion in the mouse Am J Physiol 182 390 1955
104 Neale F C The demonstration of the iron binding globulin (transferrin) in the serum and urine proteins by use of $^{59}$Fe combined with paper electrophoresis J Clin Path 8 334 1955
105 Cartwright G E Gubler C J and Wintrobe M M Studies on copper metabolism XI Copper and iron metabolism in the nephrotic syndrome J Clin Invest 33 685 1954
106 McCance R A and Widdowson E M Absorption and excretion of iron Lancet 2 680 1937
107 Hahn P F Bale W F Ross J F Balfour W M and Whipple G H Radioactive iron absorption by gastro intestinal tract influence of anemia anoxia and antecedent feeding Distribution in growing dogs J Exper Med 78 169 1943

108 Stewart W B Yuile C L Claiborne H A Snowman R T and Whipple G H Radioiron absorption in anemic dogs fluctuations in mucosal block and evidence for gradient of absorption in gastro intestinal tract J Exper Med 92 375 1950

109 Heilmeyer L Keiderling W and Wohler F Existiert bei der Eisenresorption im Dunndarm ein Mucosablock? Klin Wchnschr 35 690 1957

110 Wohler F Heilmeyer L Emrich D and Kang Shintto Zur Funktion des Ferritins bei der Eisenresorption Arch exper Path u Pharmakol 230 107 1957

111 Reissman K R Coleman T J Budai B S and Moriarty L R Acute intestinal iron intoxication Serum iron and autopsy findings Blood 10 35 1955

112 Brown E B Dubach R and Moore C V Studies in iron transportation and metabolism XI Critical analysis of mucosal block by large doses of inorganic iron in human subjects J Lab & Clin Med 52 335 1958

113 Bothwell T H Pirzio Biroli G and Finch C A Iron absorption I Factors influencing absorption J Lab & Clin Med 51 24 1958

114 Peterson R E and Ettinger R H Radioactive iron absorption in siderosis (hemochromatosis) of the liver Am J Med 15 518 1953

115 Bothwell T H van Doorn Wittkampf H van W DuPreez M L and Alper T The absorption of iron Radioiron studies in idiopathic hemochromatosis malnutritional cytosiderosis and transfusional hemochromatosis J Lab & Clin Med 41 836 1953

116 Heilmeyer L Neuere Ergebnisse der Eisenstoffwechselforschung bei der Hamochromatose Deutsche med Wchnschr 78 ?80 1954

117 Pirzio-Biroli G Bothwell T H and Finch C A Iron absorption II The absorption of radioiron administered with a standard meal in man J Lab & Clin Med 51 37 1958

118 Fontes G and Thivolle L Recherches expérimentales sur la thérapeutique de l anémie grave par carence martiale et notamment par hémorragie la reconstitution des réserves martiales Sang 10 144 1936

119 Stewart W B Vassar P S and Stone R S Iron absorption in dogs during anemia due to acetylphenylhydrazine J Clin Invest 32 1[illegible] 1953

120 Kaldor I Studies in intermediary iron metabolism VI The absorption and storage of iron in experimental anemia Australian J Exper Biol & M Sc 32 801 1954

121 Brown E B Dubach R Smith D E Reynafarje C and Moore C V Studies in iron transportation and metabolism Long term studies of iron overload in dogs J Lab & Clin Med 50 86? 1957

122 Brown E B and Justus B W *In vitro* absorption of radioiron by everted pouches of rat intestine Am J Physiol 194 319 1958

123 Wack J P and Wyatt J P Studies on ferrodynamics I Gastrointestinal absorption of Fe in the rat under differing dietary states A M A Arch Path 67 ?7 1959

124 Peterson R E and Mann J D Transport of radioactive iron in intestinal lymph Am J Physiol 169 ?63 19[illegible]

125 Everett N B Garrett W E and Simmons B S Lymphatics in iron absorption and transport Am J Physiol 178 45 19?4

126 Josephs H W Absorption of iron as a problem in human physiology A critical review Blood 13 1 19?8

127 Smith M D and Pannacciulli I M Absorption of inorganic iron from graded doses its significance in relation to iron absorption tests and the mucosal block theory Brit J Hemat 4 428 1958

128 Moore C V The importance of nutritional factors in the pathogenesis of iron deficiency anemia Am J Clin Nutrition 3 3 1955

129 Chodos R B Ross J F Apt L Pollycove M and Halkett J A E The absorption of radioiron labeled foods and iron salts in normal and iron deficient subjects and in idiopathic hemochromatosis J Clin Invest 36 314 1957

130 Hegsted D M Finch C A and Kinney T D The influence of diet on iron

absorption I The interrelation of iron and phosphorus J Exper Med 90 147 1949
131 Taylor J Stiven D and Reid L W Experimental and idiopathic siderosis in cats J Path & Bact 41 397 1935
132 Kinney T D Kaufman N and Klavins J Effect of ethionine induced pancreatic damage on iron absorption J Exper Med 102 151 1955
133 Reynafarje C Lozano R and Valdivieso J *Iron Metabolism in the Polycythemia of High Altitudes* Air University School of Aviation Medicine Bull USAF Randolph AFB Texas 1958
134 Vassar P S and Taylor D M Effects of hypoxia on iron absorption in rats Proc Soc Exper Biol & Med 93 504 1956
135 Gubler C J Cartwright G E and Wintrobe M M Anemia of infection effect of infection on absorption and storage of iron by rat J Biol Chem 184 563 1950
136 Gubler C J Cartwright G E and Wintrobe M M The effect of pyridoxine deficiency on the absorption of iron by the rat J Biol Chem 178 939 1949
137 Yuile C L Hayden J W Bush J A Tesluk H and Stewart W B Plasma iron and saturation of plasma iron binding protein in dogs as related to the gastro intestinal absorption of radioiron J Exper Med 92 367 1950
138 Venkatachalan P S Brading I Kaldor I and George E I Studies on intermediary iron metabolism XI The source of iron in the regenerated liver of the rat after hepatectomy Australian J Exper Biol & M Sc 35 353 1957
139 Balfour W M Hahn P F Bale W F Pommerenke W T and Whipple G H Radioactive iron absorption in clinical conditions normal pregnancy anemia and hemochromatosis J Exper Med 76 15 1942
140 Hahn P F Carothers E L Darby W J Martin M Sheppard C W Cannon R U Beam A S Densen P M Peterson J C and McClellan J S Iron metabolism in human pregnancy as studied with the radioactive isotope $Fe^{59}$ Am J Obst & Gynec 61 477 1951
141 Darby W J Hahn P F Koster M M Steinkamp R C Densen P M and Cook M B The absorption of radioactive iron by children 7 to 10 years of age J Nutrition 33 107 1947
142 Smith N J and Schulz J The absorption of iron in infants and children in *Iron in Clinical Medicine* edited by R O Wallerstein and S R Mettier p 65 University of California Press Berkeley Calif 1958
143 Sturgeon P Iron metabolism A review Pediatrics 18 267 1956
144 Finch C A Body iron exchange in man J Clin Invest 38 392 1959
145 Stevens A R White P L Hegsted D M and Finch C A Iron excretion in the mouse J Biol Chem 203 161 1953
146 Klein J R Metabolism of iron in the duck Am J Physiol 186 656 1959
147 Huff R L Hennessey T G Austin R F Garcia J F Roberts B M and Lawrence J H Plasma and red cell iron turnover in normal subjects and in patients having various hematopoietic disorders J Clin Invest 29 1041 1950
148 Sharney L Schwartz L Wasserman L R Port S and Leavitt D Pool systems in iron metabolism with special reference to polycythemia vera Proc Soc Exper Biol & Med 87 489 1954
149 Huff R L and Judd O J Kinetics of iron metabolism Advances Biol & M Phys 4 223 1956
150 Pollycove M Iron kinetics in *Iron in Clinical Medicine* edited by R O Wallerstein and S R Mettier p 43 University of California Press Berkeley Calif 1958
151 Alper T Savage D V and Bothwell T H Radio iron studies in a case of hemochromatosis J Lab & Clin Med 37 665 1951
152 Bothwell T H Ellis B C van Doorn Wittkampt H and Abraham O L Radioiron studies in hemochromatosis The effects of repeated phlebotomies J Lab & Clin Med 46 167 1955
153 Bothwell T H Hurtado A V Donohue D M and Finch C A Erythrokinetics IV The plasma iron turnover as a measure of erythropoiesis Blood 12 409 1957

154 Wack J P and Wyatt J P Studies on ferrodynamics II Uptake and distribution of labeled Fe⁵⁹ in experimental infusional siderosis A M A Arch Path 67 243 1959
155 Finch C A Hegsted D M Kinney T D Thomas E D Rath C E Haskins D Finch S and Fluharty R C Iron metabolism Pathophysiology of iron storage Blood 5 983 1950
156 Kinney T D Hegsted D M and Finch C A The influences of diet on iron absorption I The pathology of iron excess J Exper Med 90 137 1949
157 Nissim J A Iron storage in the adrenal cortex and medulla and cortical cell damage following the administration of different iron preparations J Physiol 119 1 1952
158 Nissim J Experimental siderosis a study of the distribution delayed effects and metabolism of massive amounts of various iron preparations J Path & Bact 66 185 19 3
159 Wyatt J P and Howell J Experimental induction of iron overload in the rat I Morphological alterations due to dietary siderosis A M A Arch Path 55 466 1953
160 Rather L J Hemochromatosis and hemosiderosis Am J Med 21 857 1956
161 Goldberg I Smith J P and Martin L E The effects of intensive and prolonged administration of iron parenterally in animals Brit J Exper Path 38 297 1957
162 Gillman T Hathorn M and Canham P A S Experimental dietary siderosis Am J Path 35 349 1959
163 Cappell S F Hutchison H E and Jowett M Transfusional siderosis The effects of excessive iron deposits on the tissues J Path & Bact 74 24 1957
164 Brown E G Smith D E Dubach R and Moore C V Lethal iron overload in dogs J Lab & Clin Med 53 591 1959
165 Beyers M R and Gitlow S E Metabolism of iron in hemochromatosis Am J Clin Path 21 349 1951
166 Davis W D and Arrowsmith W R The effect of repeated phlebotomies in hemochromatosis J Lab & Clin Med 39 526 1952
167 Davis W D Jr and Arrowsmith W R The treatment of hemochromatosis by massive venesection Ann Int Med 39 723 1953
168 Blackburn C R B McGuiness A E and Kaldor I Removal of excess body iron in haemochromatosis by repeated venesection Australian Ann Med 2 20? 1953
169 Davey D A Boxell W H and Kemp T A Treatment of haemochromatosis by repeated venesection Brit M J 2 1511 1954
170 Warthin T A Peterson E W and Barr J H Jr The treatment of idiopathic hemochromatosis by repeated phlebotomy Ann Int Med 38 1066 1953
171 Macgregor A G and Ramsay W N M Iron metabolism during treatment of idiopathic haemochromatosis Lancet 2 1314 1957
172 McAllen P M Coghill N F and Lubran M The treatment of haemochromatosis Quart J Med 26 251 1957
173 Crosbey W H Treatment of haemochromatosis by energetic phlebotomy One patient s response to the letting of 55 litres of blood in 11 months Brit J Haemat 4 82 1958
174 Seven M J Gottlieb H Israel H L Reinhold J G and Rubin M N Hydroxyethylenediamene triacetic acid versenol in the treatment of hemochromatosis Am J M Sc 228 646 19 4
175 Girard M Le traitement des hémochromatoses Rev prat 8 3041 1958
176 Frisch A V Über familiare Hämochromatose Wien Arch inn Med 4 149 19
177 Boland B F and Curran L F Hemochromatosis Two atypical cases occurring in brothers J A M A 97 3 0 1931
178 Harvier P Diamatteo J and Daniel R Le diabète bronzé familial À propos de huit observations personnelles Paris méd 37 49 1947

absorption I The interrelation of iron and phosphorus J Exper Med 90 147 1949
131 Taylor J Stiven D and Reid L W Experimental and idiopathic siderosis in cats J Path & Bact 41 397 1935
132 Kinney T D Kaufman N and Klavins J Effect of ethionine induced pancreatic damage on iron absorption J Exper Med 102 151 1955
133 Reynafarje C Lozano R and Valdivieso J *Iron Metabolism in the Polycythemia of High Altitudes* Air University School of Aviation Medicine Bull USAF Randolph AFB Texas 1958
134 Vassar I S and Taylor D M Effects of hypoxia on iron absorption in rats Proc Soc Exper Biol & Med 93 501 1956
135 Gubler C J Cartwright G E and Wintrobe M M Anemia of infection effect of infection on absorption and storage of iron by rat J Biol Chem 184 563 1950
136 Gubler C J Cartwright, G E and Wintrobe M M The effect of pyridoxine deficiency on the absorption of iron by the rat J Biol Chem 178 939 1949
137 Yuile C L Hayden J W Bush J A Tesluk H, and Stewart W B Plasma iron and saturation of plasma iron binding protein in dogs as related to the gastro intestinal absorption of radioiron J Exper Med 92 367 1950
138 Venkatachalan P S Brading I Kaldor I and George E P Studies on intermediary iron metabolism XI The source of iron in the regenerated liver of the rat after hepatectomy Australian J Exper Biol & M Sc 35 353 1957
139 Balfour W M Hahn P F Bale W F Pommerenke W T and Whipple G H Radioactive iron absorption in clinical conditions normal pregnancy anemia and hemochromatosis J Exper Med 76 15 1942
140 Hahn P F Carothers E L Darby W J Martin M Sheppard C W Cannon R U Beam A S Densen P M Peterson J C and McClellan J S Iron metabolism in human pregnancy as studied with the radioactive isotope $Fe^{59}$ Am J Obst & Gynec 61 477 1951
141 Darby W J Hahn P F Koster M M Steinkamp R C Densen P M and Cook M B The absorption of radioactive iron by children 7 to 10 years of age J Nutrition 33 107 1947
142 Smith N J and Schulz J The absorption of iron in infants and children in *Iron in Clinical Medicine* edited by R O Wallerstein and S R Mettier p 65 University of California Press Berkeley Calif 1958
143 Sturgeon P Iron metabolism A review Pediatrics 18 267 1956
144 Finch C A Body iron exchange in man J Clin Invest 38 392 1959
145 Stevens A R White, P L Hegsted D M and Finch C A Iron excretion in the mouse J Biol Chem 203 161 1953
146 Klein J R Metabolism of iron in the duck Am J Physiol 196 656 1959
147 Huff R L Hennessey T G Austin R F Garcia J F Roberts B M and Lawrence J H Plasma and red cell iron turnover in normal subjects and in patients having various hematopoietic disorders J Clin Invest 29 1041 1950
148 Sharney L Schwartz L Wasserman L R Port S and Leavitt D Pool systems in iron metabolism with special reference to polycythemia vera Proc Soc Exper Biol & Med 87 489 1954
149 Huff R L and Judd O J Kinetics of iron metabolism Advances Biol & M Phys 4 223 1956
150 Pollycove M Iron kinetics in *Iron in Clinical Medicine* edited by R O Wallerstein and S R Mettier p 43 University of California Press Berkeley Calif 1958
151 Alper T Savage D V and Bothwell T H Radio iron studies in a case of hemochromatosis J Lab & Clin Med 37 665 1951
152 Bothwell T H Ellis B C van Doorn Wittkampt H and Abraham O L Radioiron studies in hemochromatosis The effects of repeated phlebotomies J Lab & Clin Med 45 167 1955
153 Bothwell T H Hurtado A V Donohue D M and Finch C A Erythrokinetics IV The plasma iron turnover as a measure of erythropoiesis Blood 12 409 1957

## Chapter 27

# Periodic Paralysis

*Jerome W Conn and David H P Streeten*

Among the many conditions that may be associated with paralysis of the skeletal muscles very few exhibit the intermittency which is the characteristic par excellence of periodic paralysis Repeated sudden bouts of flaccid muscular paralysis or weakness in persons otherwise enjoying apparently robust health constitute a striking clinical picture which is seldom likely to be confused with other conditions The disorder has excited interest in the past 70 years out of proportion to its very low incidence because of the expectation that elucidation of its pathogenesis may contribute significantly to the understanding of muscle function in general

Periodic paralysis may be defined as a disorder occurring usually in young persons characterized by attacks of weakness or paralysis that are spontaneous or in response to stimuli such as administration or endogenous release of salt retaining adrenocortical steroids Such episodes are separated by periods of apparently normal muscular strength Relaxation of the muscles predisposes to paralysis muscular activity has a protective effect When paralysis is severe it is associated with loss of tendon reflexes and loss of excitability of the muscles to electrical stimuli applied either through the motor nerves or to the muscles directly Except in a few instances late in the disease there is no clinically evident muscular atrophy No disturbance of sensation or of consciousness is present The defect responsible for the condition is genetically transmitted in most cases but also appears to occur sporadically The abnormality underlying periodic paralysis is now thought to be a disorder in the contractility of muscle resulting from electrolyte changes associated with and probably secondary to intermittent hyperaldosteronism,

## HISTORICAL LANDMARKS

The literature prior to 1882 contains a few reports of patients who experienced repeated bouts of muscular paralysis which might well have

179 Capretti G and Telo W Emocromatosi familiare ad impronta iposurrenalica contributo clinico Gior clin med 30 857 1949
180 Lawrence R D Haemochromatosis in three families and in a woman Lancet 1 736 1949
181 Rogers W F Jr Familial hemochromatosis with comments on adrenal function in hemochromatosis Am J M Sc 220 530 1950
182 Houston J C and Zilkha K J Hemochromatosis in a family Guy s Hosp Rep 104 262 1955
183 Kappeler von R. Familiare Hämochromatose Schweiz med Wchnschr 86 477 1956
184 Lamy M and Frezal J L hémochromatose maladie génétique Bull et mém Soc méd hôp Paris 74 16 1958
185 Pirart J and Gatez P L Étiologie de l hémochromatose non transfusionnelle Semaine hôp Paris 1 1044 1958
186 Debré R Dreyfus J Frezal J Labie D Lamy M Maroteaux I Schapira F and Schapira G Genetics of haemochromatosis Ann Human Genet 23 16 1958
187 Darnis F L Hémochromatose idiopathique familiale Rev prat 8 2985 1958
188 Lohr K and Reinwein H Konkordantes Auftreten von Lebercirrhose und Diabetes Mellitus (Hamochromatose) bei eineugen Zwillingen Deut Arch klin Med 200 53 1952
189 Lesieur A À propos d un cas familial de cirrhose bronzée Arch mal app digest 39 960 1950
190 Nussbaumer T Plattner H C and Rywlin A Hemochromatose juvenile chez trois soeurs et un frère avec consanguinité des parents étude anatomo-clinique et génétique du syndrome endocrino hépatomyocardique J génét hum Geneva 1 53 1953
191 Conte J Ristelhueber J and Malvezin J C Les hémochromatoses familiales et héréditaires Bull et mem Soc méd hôp Paris 74 267 1958
192 Debré R Métabolisme du fer chez les descendants de malades atteints de cirrhose bronzé Bull et mém Soc méd Paris 68 665 1952

disease could be associated with periodic paralysis He demonstrated a greatly increased frequency of attacks during chronic administration of desiccated thyroid to patients with periodic paralysis even in the absence of any known abnormalities of the thyroid gland

The electrolyte metabolic era was foreshadowed by the report of Biemond and Daniels [12] of a patient with familial periodic paralysis in whom the serum potassium concentration was found to be 13 38 mg per 100 ml (3 4 mEq per liter) during a mild attack and 17 87 mg per 100 ml (4 6 mEq per liter) when the attack had subsided It was not until 1937 however, that Aitken and associates [13] established conclusively that both spontaneous attacks of periodic paralysis and attacks induced by administration of glucose and insulin were regularly associated with a fall in the level of serum potassium This observation provided a rationale for the therapeutic use of large doses (12 gm) of potassium salts which they employed with great effect in several attacks (Previously smaller doses had been given empirically by others [14 15]) Balance studies [16-18] demonstrated clearly that the fall in serum potassium level during attacks was not attributable to a negative potassium balance since urinary losses of this ion did not increase before attacks and fell sharply during them Studies of the movements of potassium in and out of the extracellular fluid [19 20] suggested strongly that the fall in serum potassium level during attacks results from an excessive internal shift of potassium out of the extracellular fluid probably into the muscle

The finding that deoxycorticosterone acetate and ACTH would precipitate severe paralytic attacks in patients with periodic paralysis gave rise to the suggestion that the adrenocortical steroids might be implicated in the pathogenesis of this disorder [21 22] Studies of adrenocortical function in periodic paralysis by Conn et al [23 24] have confirmed this suggestion by revealing a large increase in aldosterone excretion associated with sodium retention before attacks The retention of sodium was thought to play an important role in inducing paralysis since patients could consistently be protected from attacks by depleting them of sodium and reducing their sodium intake to a minimum The significance of these and other more recent observations [25] and their relationship to the observed potassium exchanges and electrolyte composition of skeletal muscle will be discussed in this chapter

Within the past few years it has become evident that a group of patients previously considered to be suffering from periodic paralysis differ in certain clinical and biochemical respects from patients with the classical syndrome These patients [26-43] now classified as having adynamia episodica hereditaria will be omitted from consideration in the account which follows and discussed in Chap 28

A number of excellent reviews of the literature on periodic paralysis

been attacks of what is now known as periodic paralysis Paucity of the clinical data reported and the nature of some of the recorded observations make the evidence inconclusive however It is impossible to be certain for instance that the paralysis in some of these patients was not hysterical in origin [1] or that the disorder which in other patients was associated with malaria [2-4] was actually periodic paralysis

The first report of a patient who indisputably had periodic paralysis was that of Shakhnovitch [5] in 1882 The patient was a man 44 years of age who had suffered from intermittent paraplegia for 25 years and whose father s death at 54 had been attributed to similar attacks of paralysis Shakhnovitch also described for the first time the powerful build and splendid general health of the patient between attacks the induction of attacks by cold the almost invariable onset of attacks during sleep the loss of tendon reflexes and the lack of any disturbance of speech sensory functions or control of sphincters during the episodes of paralysis His patient was considered to be suffering from a new form of neurosis for which he proposed the term *paraplegia spinalis intermittens nervosa*

The observations of Westphal [6] in 1885 established that paralysis was associated with complete loss of muscular contractility in response to both direct and indirect stimulation with galvanic and faradic current

In 1890 Goldflam [7] described the frequent occurrence of attacks on one day of the week (Friday in his case) the severe thirst which occurred during paralysis and the profuse sweating which becomes obvious towards the end of the seizures He drew attention to the tendency for the paralytic episodes to become less frequent with advancing years He was also the first author [8] to observe vacuoles in microscopic sections of the skeletal muscles Goldflam s theory that autointoxication is the cause of the malady was generally accepted for 30 years or more The theory was based on the stubborn constipation experienced by these patients during attacks and on the toxic effect of organic constituents of their urine when injected into rabbits Thus while the clinical picture had been well delineated and the underlying histopathologic changes in the skeletal muscle had been described in great detail by the end of the nineteenth century little progress had been made in tracing the pathogenesis of periodic paralysis

Between 1900 and 1937 the main contribution to an understanding of periodic paralysis was the introduction of the experimental approach to the disease This followed the discovery by Kramer [9] that the precipitation of attacks by the ingestion of large meals was due to their high content of carbohydrate This observation was independently confirmed by Shinosaki [10] who was the first to use administration of glucose for the experimental induction of paralytic attacks In company with other authors Shinosaki also confirmed Kitamura s [11] report that Graves

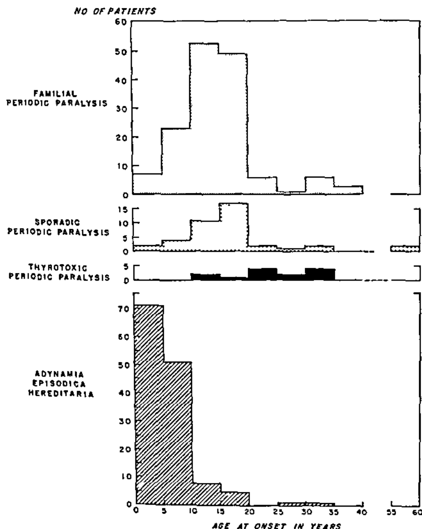



Fig 27 1 Age of onset of attacks in reported cases of familial sporadic and thyrotoxic periodic paralysis and in adynamia episodica hereditaria

to be more severe and more apt to end fatally in males than in females [48 56 62 63]

**Geographic Distribution and Racial Incidence** Although the disease is rare it has been reported from many parts of the world Most of the patients have been Caucasians from Europe and North America There have been a number of reports from Russia Japan India and Brazil No cases have been described among the Chinese or the Negro people

have been published Among the best are those of Goldflam [7 44] Taylor [45] Schmidt [46] Janota and Weber [47], Dalinghaus [48] and Talbott [49]

## CLINICAL ASPECTS

*Incidence*

**Age** The age of onset of paralytic episodes has a wide range of variation the extremes being 1 to 2 years [50] and 56 years of age [9 51] Attacks frequently begin during infancy in patients belonging to the group of adynamia episodica hereditaria (see Chap 28) and during the third or fourth decades of life in patients with thyrotoxicosis [52-55] After excluding these two groups from consideration, it appears that attacks have begun between the ages of 7 and 21 years in 93 of 100 patients described in the literature These features are shown in Fig 27 1 which gives the distribution of the ages at which paralytic episodes first occurred in the published reports of periodic paralysis (familial sporadic and thyrotoxic) and of adynamia episodica hereditaria The similarity in age of onset in familial and sporadic cases of periodic paralysis is in good accord with other evidence that the two conditions are identical except for the evident lack of hereditary transmission in the sporadic group The striking differences between periodic paralysis and adynamia episodica hereditaria on the other hand support the view that these are different entities

Several reports show a striking constancy in the age of onset of paralysis among most members of an affected family e g 7 to 8 years in the family of Biemond and Daniels [12] 12 to 16 years in one of Mankowsky s families [56] and 17 to 19 years in MacLachlan s [57] and Khan s [58] families

The severity and frequency of attacks usually increased to a maximum at about 20 years of age remained substantially unchanged for 10 years or more, and became less severe and less frequent especially in females from about the age of 30 to 40 years frequently disappearing some time between the ages of 32 and 73 years [7 13 17 46 56 59] A number of patients did not follow this usual benign course Some persons continued to experience severe attacks in middle life or old age [7] In a few of the larger families progressive muscular atrophy with permanent weakness or paralysis supervened in a number of the elderly patients [12 60 61]

**Sex** In most families with periodic paralysis the disorder occurred more frequently among males than among females The preponderance of males was slight in some families (Table 27 1a) and overwhelming in others (Table 27 1b) Among patients with thyrotoxicosis, males have been exclusively affected (Table 27 1c) The vast majority of sporadic cases have also been males

Both in familial and in sporadic cases attacks of paralysis have tended

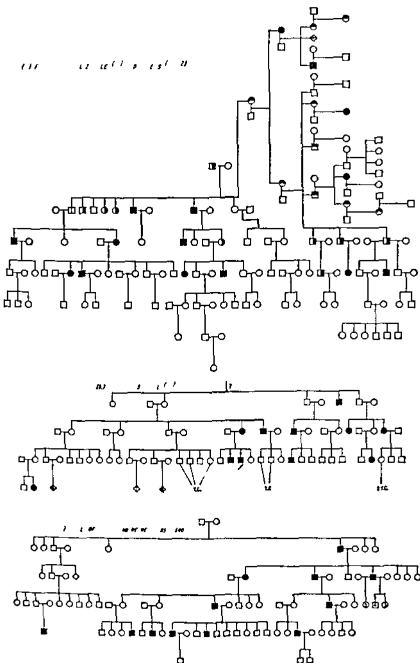


Fig. ?-? Genealogy of periodic paralysis in three families (See Fig. ?-3 for explanation of symbol.)

TABLE 27 1 SEX INCIDENCE OF OVERT PERIODIC PARALYSIS IN A FEW OF THE REPORTED FAMILIES

| Type of case | No of cases | | Reference |
|---|---|---|---|
| | Males | Females | |
| a Familial euthyroid cases with no marked male preponderance | 8 | 6 | [1?] |
| | 6 | 5 | [44] |
| | 6 | 5 | [4 ] |
| b Familial euthyroid cases with marked male preponderance | 11 | 1 | ['0] |
| | 11 | 3 | [56] |
| | 5 | 0 | [4?] |
| | 8 | 0 | [ 8] |
| | 8 | 1 | [ ?] |
| c Patients with thyrotoxicosis | 1 | 0 | [9?] |
| | 4 | 0 | [53] |
| | 1 | 0 | [54] |
| | 1 | 0 | [118] |
| | 6 | 0 | [8?] |
| | 1 | 0 | [55] |
| | 3 | 0 | [94] |

and Dalinghaus [48] has drawn attention to the absence of the condition in the tropics

*Condition between Attacks*

Between attacks most patients have been free of symptoms and have had no abnormal physical findings The one striking feature that many authors have commented upon has been the robust physique and the appearance of unusually good muscular development in most of the males [5 9 17, 46 47, 64–74] Even those of asthenic habitus or short stature [70] usually had an athletic build The muscular size has at times suggested pseudohypertrophic muscular dystrophy, and muscular strength was sometimes less than normal [46] More frequently, however the strength of the muscles was as much increased as their size so that the patients were good athletes who enjoyed a rugged outdoor life [47 61 75]

It has been reported that Chvostek s sign was present between attacks [76, 77] and disappeared during attacks in three patients [77] and that it remained positive in a fourth [76] Goldflam [7] found reduced electrical excitability of the muscles between attacks in two patients but Janota and Weber [47] considered that the electrical excitability of the muscles to direct and indirect stimuli was slightly increased between attacks

*Associated Disorders*

In some patients the clinical features of thyrotoxicosis have been present with enlargement of the thyroid gland and laboratory evidence

due to the high carbohydrate content of such a meal [9] administration of glucose has been used as an experimental method of precipitating attacks [10] Insulin alone will induce attacks [87] and the administration of both glucose and insulin is a very effective method of precipitating attacks [13 16 21 23 24 59 86 89 93-96]

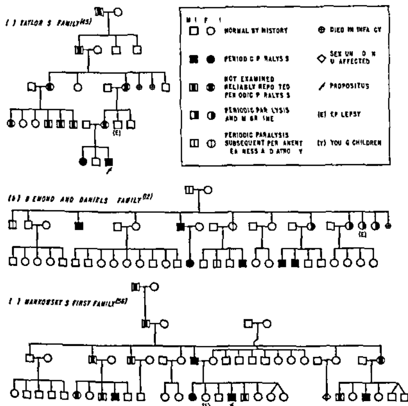



Fig 27 3 Genealogy of periodic paralysis in three families

**Rest and Muscular Relaxation** Rest especially following vigorous exertion appears to play an important role in precipitating attacks Thus many writers [5 7 9 10 12 14-16 18 45-47 49 52 53 57-60 62 64 65 68 70-73 78 80 82 85 88 90 92 94 96-110] have mentioned that the severe attacks usually occur at night in the early hours of the morning or when the subject is at rest during the day Conversely patients frequently learn that they can abort mild attacks ( walk them off ) by exercise [15 16 41 44 53 69 70 100 102] That rest may have local effects on the muscles of these patients has been shown by the observation that paralysis may supervene in an immobile limb while active limbs escape [44 70 75] The strong tendency for attacks to recur on the same

of hyperthyroidism (elevated basal metabolic rate (BMR), increased uptake of radioactive iodine by the thyroid and raised serum protein bound iodine (PBI) concentration) In these persons paralytic episodes started after the onset of thyrotoxicosis Shinosaki [10] and other Japanese authors have emphasized that the thyroid is frequently enlarged without evidence of hyperfunction of the gland but these observations were made before the advent of modern methods of appraisal of thyroid function

Various *neurologic disorders* may occur together with periodic paralysis *Migraine* is the most important of these A history of migraine in patients with periodic paralysis and in their relatives has been described frequently [12, 15, 47, 56 57, 59–61, 68 70 71, 78–80 cf also 81] Migrainous headaches and paralytic attacks may occur in the same patient at different times or simultaneously and the occurrence of a headache has even seemed to protect some patients temporarily from an attack of paralysis acting as a type of 'equivalent for attacks of periodic paralysis [52 60] Gardner [68] MacLachlan [57] and Holtzapple [60] have all described the occurrence of headaches for several years followed in the same patient by the onset of paralytic attacks and cessation of the headaches Of the 17 patients with periodic paralysis in Holtzapple s family [60] the disease was transmitted through a parent with paralytic attacks in 10 instances and through a parent who had frequent sick headaches in the remaining 7 instances (Fig 27 2*a*) If migraine and periodic paralysis constitute different modes of expression of the same abnormal gene as some of the evidence might suggest (see Genetics further on) then the incompleteness of penetrance in this family—and possibly in some of the others in the literature—would be more apparent than real *Epileptic seizures* have been reported frequently in patients with periodic paralysis [5, 56 72 87] and in some of their unaffected relatives (Fig 27 3) *Progressive muscular atrophy* on the other hand apparently develops mainly in patients who have experienced paralytic episodes for many years and not in unaffected relatives [12, 59–61] (Figs 27 2*a* and 27-3*b*)

*Factors Which Induce Attacks*

Four groups of stimuli are known to precipitate attacks of paralysis in patients with the disease

**"Stresses" of a Wide Variety** These have included infectious diseases [6 57, 60 83] surgical and accidental trauma [62 83 84] emotional excitement anxiety or anger [10 15, 16 22 45 59 70 85] severe muscular exertion [10 45 68 78 80 86 87] menstruation [15, 88] and exposure to cold [5 46 47 57–59 89–91]

**Increased Utilization of Carbohydrate** That the ingestion of a heavy meal particularly in the evening tends to precipitate an attack has long been recognized [7 15 16 58 59 65 68 71 72 91 92] Drinking beer has also been incriminated [68 86 91] Since the effects of a heavy meal are

overcome by actively exercising the affected muscles [*5 7 10 16 41 53 59 70 100 102*]

The muscles may be painful during attacks [*90*] but the pain may often be relieved by a change of position [*52*] Several authors have been struck by the swelling of the affected muscles which gave them a firm rubbery consistency during attacks [*5 57 59 69 71 83 85 89 105*] Measurement of the affected limbs has shown that the apparent swelling has frequently been associated with an increase in circumference during paralysis [*69 72 90 110 113*] Contraction of the affected muscles on direct or indirect stimulation with galvanic or faradic current is reduced or completely abolished in proportion to the severity of the paralysis [*4 6 7 13-15 68, 87 106*] The deep tendon reflexes are reduced or abolished but the abdominal and cremasteric reflexes may be unaffected There is no loss of any sensory modality and no impairment of mental faculties or consciousness during even the severest of attacks

The myocardium may be affected along with the skeletal muscles during paralytic seizures Oppenheim's [*106*] original description of transient bradycardia and cardiac dilatation with the appearance of a systolic murmur at the apex believed to result from mitral insufficiency during attacks has been confirmed in part or entirely by many observers The bradycardia which is typical of the fully developed attack [*68 90 95 106*] is preceded by tachycardia before and early in the attack [*14 46 47*] The clinical evidence of transient cardiomegaly [*10 46 66 68 106*] has been verified by x ray [*21 77 113*] Elevation of blood pressure [*10 46 66 110 114 116*] and arrhythmias [*10 44 80 103*] have been reported during attacks

Gastrointestinal features of the attacks include occasional nausea and vomiting [*46 68 77 78 95 102*] and constipation bowel movements occurring seldom if ever during severe attacks [*7 12 46 66 71 80 100 116*] The possibility that these findings might result from loss of normal intestinal peristaltic motility was suggested to Dalinghaus [*48*] by the reports that bowel sounds are absent during attacks [*46 80*]

Changes in the intake and urinary output of water or both have frequently been described Thus severe thirst and increased imbibition of fluids may occur before or early in attacks [*7 10 12 16 47 72 106 116*] In contrast whether fluid intake is increased or decreased urine is seldom passed spontaneously during an attack [*12 14 45-47 66 69 71 74 77 78 89 90 100*] When the urine volume has been measured in relation to the fluid intake positive water balance has been found during paralysis [*49*] Severe sweating may occur during paralytic seizures and usually tends to usher in the phase of recovery [*6 7 10 12 14 46 47 57 69-72 84 90 100 106*]

**Phase of Recovery** The commencement of improvement is frequently evident clinically by the occurrence of a bout of severe sweating and by

day of the week, usually on Sundays or Mondays [7 10, 15, 65 68 73, 90] may be related to indulgence in more relaxation and a greater intake of food during the week end, or in some cases to increased athletic activity in the presleeping hours

**Drugs** Drugs which have been used to stimulate attacks include epinephrine [10 16, 59, 87, 95, 110–112] desiccated thyroid [10 15 87] parathyroid extracts [10] adrenocortical extracts [95] deoxycorticosterone acetate [21 25] ACTH [22] 2α methyl 9α fluorohydrocortisone [24] and ammonium glycyrrhizinate [25]

*Prodromal Symptoms*

While many patients are unaware of an impending attack until they awake paralyzed in the early hours of the morning some patients have experienced recognizable prodromata The commonest appear to have been sensations arising from the muscles, described variously as aches and pains or cramps in the muscles [6 10, 14 16, 53 68 90, 100 10?] stiffness or a tight feeling [15 57 71 86 93] and fatigue [9, 10 52 60 72 98] Other patients have noticed numbness tingling and other paresthesias [6, 55 60 71 75 78] apprehension irritability or excitability [65 67 72], and an increase in thirst preceding attacks [9 16 45, 71, 72]

*Paralytic Attacks*

Paralytic attacks may occur from once in a lifetime [47] to once a week [7 10, 15 65, 68 73 90] there may even be nightly attacks for 10 years [71]

Abortive attacks may last for less than an hour while attacks with complete paralysis usually last 6 to 24 hr More severe seizures may endure for 2 or 3 days while Donath [84] and MacLachlan [57] have both recorded authentic instances of attacks lasting 8 days

**General Description of Attacks** In attacks which develop while the subject is awake weakness and then paralysis usually appear first in the lower limbs then in the arms trunk and neck in that order [6 7 14] More characteristically patients are awakened in the early hours of the morning with widespread flaccid paralysis which renders them helpless

The diaphragm the muscles of the face and mouth and those involved in speech and swallowing are spared with rare exceptions such as in Putnam s [65] patient who in some of his severe seizures could move only his lips and eyes On the rare occasions when death has ensued it has usually occurred during an attack involving the muscles of respiration [57] (see Mortality and Pathologic Findings further on in this chapter)

Many attacks are only partial involving a few muscle groups a single extremity or two limbs on the same side of the body or opposite sides—i e , hemiplegia or paraplegia [95] Minor attacks of localized weakness may occur during the day it is these attacks which can frequently be

sistent and is of doubtful significance On two occasions endogenous creatinine clearance showed a slight reduction of questionable significance on the day of an attack [117 122]

*Urinary creatine* has been reported increased between attacks [95] to fall before the day of paralysis [21] and to rise during attacks [18 100]

*Urinary excretion of phosphorus* tends to be reduced in attacks roughly in parallel with urinary potassium [16] An increased output of phosphorus after the attacks [16 123] is associated with no increase in the output of calcium and is therefore probably derived from muscle rather than from bone [123]

*Excretion of calcium and magnesium* has been found to be increased during attacks [10 18]

Changes in urinary excretion of sodium potassium chloride nitrogen uric acid pepsinogen 17 hydroxycorticoids 17 ketosteroids and aldosterone will be considered in detail in a subsequent section (see Metabolic Studies further on)

## BIOCHEMICAL CHANGES IN BLOOD

The *fall in serum potassium* [12 13 16 21 23 49 65 69 74 94–96 98 119] and the *rise in serum sodium* concentration [23 24] will be discussed more fully later (see Metabolic Studies below)

Serum *inorganic phosphorus* falls for a few hours This was reported first by Aitken et al [13] and was confirmed by many others [16 21 74 98 115] The fall per se is probably of little or no importance in paralyzing the muscles since the infusion of sodium phosphate does not prevent paralysis [115]

*Blood cholesterol* concentration usually rises during attacks of paralysis [119a]

No change has been reported in *blood pH* [69] *blood urea* [13 103] *serum calcium* [10 13 18 21, 80] or *serum magnesium* [13 18 21] although Yoshimura [124] found a rise in serum magnesium during attacks Gass et al [103] reported that the cephalin flocculation and thymol turbidity tests icteric index and serum total proteins were normal in their patient

## ELECTROMYOGRAPHIC AND CHEMICAL CHANGES IN MUSCLE

### Electromyographic Findings

These have been described in detail in two patients with periodic paralysis by Lichler Jantz and Jung [96] In the intervals between attacks the biphasic muscle action potentials were found to be normal in duration and shape Rapid faradic stimulation however caused rhythmic fluctuations in the amplitude of the action potentials These were more pronounced than the rhythmic changes seen in normal subjects indicating

the onset of diuresis [24, 46, 49 66] Muscles which were paralyzed first are usually the last to recover their function strength returning first to the neck and arms Recovery is usually complete in 1 to 3 hr from its commencement but it may take a day or more Pain stiffness or soreness in the muscles may persist for a day or two after the attack [60, 71 108] Patients customarily lose 1 to 3 kg in body weight because of the diuresis which occurs during recovery [117]

## LABORATORY FINDINGS

### ROUTINE LABORATORY FINDINGS

**Urinalysis** Transient mild proteinuria [9, 10 12, 46 47, 56] glycosuria [9 10] acetonuria [10 47, 118] and cylindruria [118] have been described during the attacks

**Hematologic Changes** Goldflam [8] reported a consistent neutrophil leukocytosis with eosinopenia during the attacks and lymphocytosis with eosinophilia after the attacks These findings have been confirmed by other authors [14 56, 66] Sugimoto [92] described the occurrence of lymphopenia during attacks of paralysis in a thyrotoxic patient

**Electrocardiogram** The ECG is normal between attacks and shows the changes characteristic of hypokalemia during episodes of paralysis viz flattened or inverted T waves particularly in the chest leads depression of RS-T segment and the occurrence of large U waves giving rise to the appearance of a prolonged Q T interval [19 21 24, 47 55 59 71 74 80 96 102, 108 109 119] Grob et al [19] have pointed out that the hypokalemic changes in the ECG are more severe in these patients than in normal persons at the same level of serum potassium

**Cerebrospinal Fluid** It may contain a slightly increased amount of protein [80 103] but shows no abnormality in pressure or cell count [49, 62 103] The potassium concentration in the cerebrospinal fluid is unchanged [95 103] or may be lowered [120] during attacks of paralysis

**Radiologic Abnormalities** These are restricted to the evidence of enlargement of the heart during the attacks [21 77 113] Cerny et al [114] have reported bony bridging of the sella turcica in four out of five affected members of a family with periodic paralysis

**Other Tests** Electroencephalography has shown no abnormalities [108] The BMR is elevated during paralysis contrary to the expectation in a paralyzed person [71 88 121] Gastric juice has been reported to contain little or no acid during attacks [15 48 77]

### BIOCHEMICAL CHANGES IN URINE

*Urinary excretion of creatinine* has been reported by Mitchell et al [15] to be low before attacks and high after attacks A slight fall in creatinine output [117] has been observed during attacks the finding was incon

significantly different from the findings in normal subjects in whom KCl administration was consistently followed by increased uptake without subsequent loss of potassium from the forearm tissues In most instances improvement in muscular strength following KCl administration lagged behind the rise in plasma potassium concentration but rapid improvement occurred when potassium loss from the tissues started i e in the second and third hours after KCl had been given These findings together with the observation of increased potassium uptake by the tissues even in mild attacks associated with no fall in plasma potassium concentration suggested that abnormal uptake of potassium by the muscles is more important than the fall in plasma potassium in inducing muscular weakness This suggestion is in good agreement with the conclusions of Fichler et al [96] based on electromyographic evidence It receives further support from the observation that during recovery from attacks there was loss of potassium from the forearm which was hastened by increasing muscular activity as recovery progressed

Zierler and Andres [20] made similar observations on the arteriovenous differences of potassium in two of the patients with familial periodic paralysis investigated by Grob et al [19] These authors suggested that the nocturnal onset of spontaneous attacks and their usual spontaneous recovery later in the morning might be due to an exaggeration of the normal diurnal rhythm of potassium influx into the tissues at night and potassium efflux from the tissues during the day

*Muscle Function Studies*

The investigations of Grob et al [19] have shown that the muscle end plate became resistant to the depolarizing effect of intraarterially injected acetylcholine during bouts of weakness Attacks were also accompanied by reduced propagation of muscle action potentials across the muscle following a single stimulus to the ulnar nerve It was concluded that the decreased responsiveness of the muscles to nervous stimulation during attacks might be due to the development of resistance to the depolarizing action of acetylcholine normally released from the motor nerve endings Since an increase in the ratio $[K_{intracellular}]/[K_{extracellular}]$ ($[K_i]/[K_e]$) causes hyperpolarization in experimental animals it was suggested that a similar change in this ratio might produce hyperpolarization of the motor end plate during attacks of periodic paralysis with resulting loss of muscular responsiveness to nervous stimulation Similarly hyperpolarization of the entire muscle membrane might bring about the observed reduction in the spread of the excitation wave to other parts of the muscle fiber

The studies of Grob et al [19] confirmed the observation of Merton (reported by McArdle [41]) that in patients with periodic paralysis the force of muscular contraction was reduced more rapidly and more severely than the amplitude or the propagation of the muscle action potentials

that the muscle was abnormal even in the unparalyzed state After the administration of glucose and insulin, but before paralysis supervened progressive widening of the action potentials occurred to two or three times their normal duration Usually there was lengthening of the latent period between stimulus and response As weakness advanced to complete paralysis there was progressive increase in the latent period, while the action potentials became more prolonged in duration and reduced in amplitude, until they could no longer be elicited shortly before all voluntary contractility of the muscle was lost Rapid faradic stimuli induced a progressive increase in the rhythmic fluctuations in amplitude of action potentials until even before paralysis supervened the motor response to indirect faradization disappeared

Eichler et al [96] have pointed out that the abnormalities in muscle action potentials started before paralysis of voluntary movement had taken place and before any other objective changes such as the fall in serum potassium level or alterations in ECG at a time when the only change was a subjective feeling of weakness During the process of recovery from attacks the abnormalities in the electromyogram (EMG) were still present when the serum potassium had returned to normal or elevated levels *and these abnormalities were restored to normal only* after further delay These findings indicate that the abnormalities in muscle action potentials do not result from the lowered level of serum potassium Eichler et al suggested therefore that the EMG changes might reflect a movement of potassium into the muscle during paralysis

*Potassium Exchanges between Plasma and Tissues*

Grob et al [19] have studied arteriovenous differences in plasma potassium concentration in three patients between and during attacks of weakness and paralysis Their results indicated that the uptake of potassium from the arterial plasma by forearm tissues after administration of insulin or of epinephrine was significantly greater in the patients with periodic paralysis [19] than in the normal persons studied [19] In 15 spontaneous attacks of weakness which followed evening meals high in carbohydrate content the patients showed significant increases in potassium uptake from the arterial blood into the tissues as compared with the potassium uptake by their tissues before the attacks That the muscle and not the bone or skin was the site of the increased uptake of potassium during the attacks is suggested by the finding that the weakened contractions which could still be elicited during the attacks resulted in an increase in venous plasma potassium This increase was four times as great as prior to the attack and twice as great as in normal persons following much stronger muscular contractions The administration of KCl during attacks of paralysis caused initial uptake of potassium by the tissues followed paradoxically by a loss of potassium from the forearm

TABLE 27 ? CONCENTRATIONS OF SODIUM AND POTASSIUM (IN MEQ/KG) IN SKELETAL MUSCLE IN PERIODIC PARALYSIS HEALTHY PERSONS AND I RIMARY ALDO TERONISM

| I at t | Diag | D t | P aly | M scl N | M scl K | M scl N + K | R fer |
|---|---|---|---|---|---|---|---|
| R S | P d p ly | O t 3 19 6 | 0 | 46 | 75 | 121 } | |
| R S | P d p ly | N 29 19 6 | 0 | 34 | 74 | 109 } 114 5 | |
| R S | P od i ly | O t. 31 1955 | 1+ | 48 | 82 | 130 } | |
| R S | P od p ly | J 14 195( | 3+ | 5 | 85 | 137 } m | |
| R S | P od a p ly | F 1 19 1957 | 4+ | 46 | 81 | 1 7 } 130 8 | |
| W F | P odi p aly | F b 8 1957 | 4+ | 47 | 8 | 129 } | |
| | H lthy | | 0 | 31 | 93 | 124 } m n | [173] |
| | H lthy | | 0 | 21 | 106 | 1 7 } 125 5 | [174] |
| | H lthy | | 0 | | 9 | | [175] |
| | H lthy | | 0 | 6 36 | 91 107 | | [1 6] |
| M W | Prim y ldoster m | 1955 | 0 | 49 | 6 | 111 } | [154] |
| | P m y ld t m | 1956 | 0 | 52 | 68 | 120 } | [17(] |
| J S | Prim ry ldoste m | 1955 1959 | 0 | 35 | 89 | 1 4 } | [117] |
| A F E | P m ry ald t m | 1955–1959 | 0 | 54 | 59 | 113 } | [117] |
| S W | P m ry ldost m | 1955 19 9 | 0 | 43 | 66 | 109 } m | [117] |
| R M S | P i y ll t m | 19 5 19 9 | 0 | 32 | 82 | 114 } 111 3 | [117] |
| J H | P mary ldost m | 19 5 19 9 | 0 | 31 | 83 | 114 } | [117] |
| B L | P m y ld te m | 1955 19 9 | 0 | 43 | 71 | 114 } | [117] |
| C G | Prim ry ldost m | 1955–1959 | 0 | 39 | 51 | 90 } | [117] |
| M M | P im y ll te m | 195 1959 | 0 | 30 | 74 | 104 } | [117] |

Aft 44 d y l w d m tak f ll w g t l d salt g w th ta l m l (D m ) l m re pto (Tl i m n )
N te 4+ = m l t q drupl gi 3+ = se p ly f th l mbs 2+ = w k ess f m d legs 1+ = m ll w k ess f ms legs

high sodium content of the muscles probably predisposed the subjects to paraly is since reduction of the sodium concentration by the desalting rendered the muscles immune to the paralytic effects of glucose and insulin and of mineralocorticoids (see under Sodium further on)

Paralyzed or weakened muscle consi tently showed higher potassium concentrations (82 85 81 82 mEq per kg) th n muscle sampled between attacks (75 74 mEq per kg) These results suggest that an acute shift of potas ium may have taken place into muscle during the process of becoming paralyzed a conclusion in good accord with the other evidence described above [19–21 105] In uch paralyzed mu cle the sodium concentration remains high and the potas ium concentration is increased so that the concentration of (Na + K) becomes con istently higher

following nervous stimulation For this reason it was postulated that the most important defect in periodic paralysis might lie in the contractility of the muscle itself rather than in transmission of the nervous impulse to the muscle Again the hyperpolarization of the entire muscle fiber resulting from increase in [K]/[K] might be incriminated as an important defect in the muscular contractility, since actomyosin is kept in the uncontracted state by high membrane potentials [126]

*Chemical Composition of Skeletal Muscle*

Evidence obtained by chemical analysis of muscle obtained by biopsy lends support to the more indirect evidence of potassium shifts in periodic paralysis described above Jantz [21] reported that the concentration of potassium in muscle was increased at the height of an attack of paralysis to 710 mg per 100 ml (182 mEq per kg), and fell within half an hour of the recovery of normal muscle function to an approximately normal figure 360 mg per 100 ml (92 mEq per kg) Vastola and Bertrand [108] found that the onset of paralysis was associated with the movement of 129 mEq of potassium into each kilogram of muscle solids together with an increase *of the water content especially in the extracellular fluid phase (i e the* chloride space) of the muscle The change in water content neutralized the effect of the potassium shift on the *concentration* of potassium in the sample of wet muscle which was unchanged by the occurrence of paralysis Since the calculations of Vastola et al [108] show that the concentration of potassium in intracellular water actually fell from 196 5 to 190 mEq per liter when paralysis supervened their contention that the resting membrane potential had increased during paralysis is difficult to accept However the increased *quantity* of potassium which these authors found within the muscle cells might be an even more important inhibitor of the association of actomyosin than an increased *concentration* of potassium [126]

More recent determinations of the electrolyte composition of muscle in periodic paralysis carried out in the authors laboratory (Table 27 ?) are in general agreement with the results of Jantz [21] Between attacks of paralysis the concentration of potassium in the muscle was low (75 mEq per kg) probably indicating chronic potassium deficiency The sodium concentration (46 mEq per kg) was considerably elevated as is usual in chronic potassium deficiency [127] In fact the muscle composition between attacks closely resembled that seen in primary aldosteronism "Desalting the patient with diuretics reduced the sodium concentration in the muscle to 34 mEq per kg without materially affecting the potassium concentration Since the sodium concentration in the muscle was high between attacks and was increased only slightly or not at all when paralysis was induced it seems likely that the supervention of paralysis did not involve a further shift of sodium into muscle On the other hand a

TABLE 9/ 9 CONCENTRATIONS OF SOI IUM AND I OTASSIUM (IN MEQ/KG) IN SKELETAL MUSCLE IN PERIODIC PARALYSIS HEALTHY PER ONS AND PRIMARY ALDOSTERONISM

| Pat nt | Diag | D t | P aly | M sel N | M sel K | M sel N + K | R f |
|---|---|---|---|---|---|---|---|
| R S | P l p ly | O t 3 19 6 | 0 | 46 | 75 | 1 1 } m | |
| R S | I od r lysı | N 9 19 6 | 0 | 34 | 74 | 109 } 114 5 | |
| R S | P od p ralysıs | O t 31 1955 | 1+ | 48 | 8 | 130 | |
| R S | P d p lysı | J 14 19 0 | 3+ | 5 | 85 | 137 m n | |
| R S | P d p ly | F l 19 19 7 | 4+ | 46 | 81 | 127 130 8 | |
| W F | P riod p ly | F b 8 1957 | 4+ | 47 | 82 | 1 9 | |
| | H lthy | | 0 | 31 | 93 | 124 } m n | [173] |
| | H lthy | | 0 | 21 | 106 | 1 7 } 125 5 | [174] |
| | H lthy | | 0 | | 95 | | [175] |
| | H lthy | | 0 | 26–36 | 91 107 | | [176] |
| M W | Prım ry ldost m | 19 5 | 0 | 49 | 62 | 111 | [154] |
| | Prım y ld t m | 1956 | 0 | 5 | 68 | 120 | [176] |
| J S | Prım ry ldoster m | 1955–1959 | 0 | 35 | 89 | 124 | [117] |
| A F E | P ry ld te nı m | 19 5–19 9 | 0 | 54 | 59 | 113 | [117] |
| S W | P m ry ldost m | 1955–19 9 | 0 | 43 | 66 | 109 | [117] |
| R M S | P m y ll te m | 19 5 1959 | 0 | 32 | 82 | 114 } m n 111 3 | [117] |
| J H | P m ry ldoste m | 1955 19 9 | 0 | 31 | 83 | 114 | [117] |
| B L | Prım y ld te m | 1955 1959 | 0 | 43 | 71 | 114 | [117] |
| G G | Prım ry ldoste nı m | 1955–19 9 | 0 | 39 | 51 | 90 | [117] |
| M M | P m y ld te m | 1955 1959 | 0 | 30 | 74 | 104 | [117] |

Aft 44 d y l w d m tak f ll w g t l d lt g th ta l m l (D m ) d m μt m (Thi m rın)
N t 4+ = mpl t q drıpl gı 3+ = se p ly f th l mt 2+ = w ak as f m d f gs 1+ = m ld k ess f m l g

high sodium content of the muscles probably predisposed the subjects to paralysis since reduction of the sodium concentration by the desalting rendered the muscles immune to the paralytic effects of glucose and insulin and of mineralocorticoids (see under Sodium further on)

Paralyzed or weakened muscle consistently showed higher potassium concentrations (82 85 81 82 mEq per kg) than muscle sampled between attacks (75 74 mEq per kg) These results suggest that an acute shift of potassium may have taken place into muscle during the process of becoming paralyzed a conclusion in good accord with the other evidence described above [19–21 108] In such paralyzed muscle the sodium concentration remains high and the potassium concentration is increased so that the concentration of (Na + K) becomes consistently higher

(130 137, 127 129 mEq per kg) than in the unparalyzed state (121 108 mEq per kg) and higher than in normal subjects and patients with primary aldosteronism Lales et al [120] have confirmed the presence of raised intracellular sodium and reduced intracellular potassium concentrations between attacks and the finding of a higher intracellular potassium concentration during an attack As suggested by Conn et al [23, 24] it is possible that the excessively high *Na + K content* of the muscles consequent upon an acute inward shift of potassium is the cause of the paralytic attacks since sodium and potassium have similar effects on the association of actomyosin [128] and since the actomyosin system is sensitive to the total *amount* of intracellular Na + K and not to their molar concentration [126]

*EXCHANGEABLE SODIUM AND POTASSIUM* (*Table 27 3*)

In four patients with periodic paralysis, Streeten and Conn [75] have found the exchangeable body sodium (Na ) to be high normal or elevated between attacks (46 6 48 3 51 3 and 51 4 mEq per kg) the normal range for Na at 24 hr in the same laboratory is 38 5 ± 2 9 (s d ) mEq per kg In

TABLE 27 3 EXCHANGEABLE SODIUM AND POTASSIUM IN PERIODIC PARALYSIS

| *Patient* | *Diagnosis* | *Na mEq/kg* | *K mEq/kg* | *Na /K* |
|---|---|---|---|---|
| R S | Periodic paralysis | 51 3 | 35 3 | 1 44 |
| W F | Periodic paralysis | 48 3 | 41 5 | 1 20 |
| F L | Periodic paralysis | 51 4 | | |
| B K | Periodic paralysis with thyrotoxicosis | 46 6 | | |
| 8 normal males (Streeten et al [75]) | | 38 5 ± 2 9 | | |
| 20 normal males (Sagild et al [129]) | | | 49 3 ± 6 4 | |
| 7 normal males (Robinson et al [131]) | | | | 0 74–0 94 |

24-hr values using $Na^{22}$ and $K^{42}$ separately

two of these patients exchangeable body potassium (K ) was determined and found to be 35 3 and 41 5 mEq per kg these values are slightly low for young males (normal mean 49 3 ± 6 4 (s d ) mEq per kg [129]) The high Na results may reflect the paucity of body fat in these lean athletic individuals but the slight lowering of K is all the more remarkable *for the same reason The patient studied* by McArdle and Merton [130] had an exchangeable body potassium (43 4 mEq per kg) that was considered normal though it was well below the value obtained in their one normal control (51 3 mEq per kg) The ratio Na /K in the authors patients (1 44 and 1 20 Table 27-3) was extraordinarily

high for lean muscular males normal values being 0 74 to 0 94 for males [*181*] Such elevation of the ratio is seen also in primary aldosteronism in conditions associated with edema and in states of severe chronic debility and inanition It has never been reported in apparently robust young males in the absence of periodic paralysis or primary aldosteronism

METABOLIC STUDIES

*Potassium*

**Serum Potassium Concentration** Between attacks the serum potassium concentration is normal in some patients but in others it may tend to be low In one patient [*75*] for instance the fasting level of serum potassium was below 4 0 mEq per liter on 26 out of 28 days between attacks and was 3 3 mEq per liter or lower on 10 of these days Repeatedly lowered serum potassium concentrations between attacks are evident also in the protocols of the patients reported by Talbott [*49*] and by Gass et al [*103*] A rapid and profound fall in serum potassium concentration, frequently to as low as 1 5 or 1 7 mEq per liter is one of the most consistent findings in attacks of periodic paralysis in patients with the familial sporadic or thyrotoxic forms of the disease [*12 13 16 21 23 49 55 59 74 94–96 98 119*] The occurrence of hypokalemia during spontaneous and induced attacks is seen in Figs 27–4 to 27 6 Several authors have drawn attention to the variability in the level of serum potassium at which paralysis occurs [*16 17 74*]

**Fate of the Disappearing Serum Potassium** In 1938 Allott and McArdle [*16*] and Pudenz et al [*95*] independently showed by the balance technique that the fall in serum potassium could not be attributed to losses of potassium in the urine or stool On the contrary urinary potassium excretion fell strikingly during the attacks as many observers have subsequently confirmed [*17 18 21–23 49 108 119*] In the absence of an increased external loss of potassium the fall in serum potassium was attributed to an internal shift from the extracellular fluid into some other compartment presumably intracellular This conclusion was supported by the electromyographic and muscle composition studies described above

**Changes in Urinary Potassium** In attacks of paralysis induced by glucose (150 gm) and insulin (20 units) or by the potent synthetic mineralocorticoid 2α methyl 9α fluorohydrocortisone (1 mg p o ) the urinary excretion of potassium declined simultaneously with the fall in serum potassium as shown in Fig 27-4 In the spontaneous attacks of paralysis too the reduction in urinary potassium output accompanied but never preceded the fall in serum potassium concentration Reduced urinary excretion of potassium is therefore probably entirely a secondary phenomenon consequent upon the fall in serum potassium which results from internal sequestration of potassium in the muscles or elsewhere It

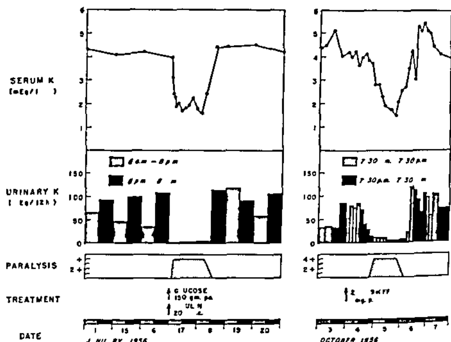



Fig 27-4 The time relationships between potassium changes in serum and urine during attacks of periodic paralysis induced by (1) glucose and insulin and (?) $2\alpha$ methyl $9\alpha$ fluorohydrocortisone

seems important that muscle function often begins to return before serum potassium values rise significantly This phenomenon is preceded by sodium diuresis After the end of the attacks diuresis of potassium usually occurred for 1 or 2 days

*Sodium*

**Sodium Retention** Severe attacks of paralysis either pontaneous or induced are usually preceded by sodium retention resulting in a positive balance of 100 mEq or more Figure 27 5 shows the urinary output of sodium over a period of 8 weeks in a patient who experienced several spontaneous attacks of weakness and paralysis during this time These included one spell of complete quadriplegia (designated as 4+ paralysis in the figure) three of severe paralysis of the limbs (3+ paralysis) one of weakness of the arms and legs (2+) and several of mild weakness of arms or legs (1+) Most of the severe attacks were preceded by sodium retention for 1 to 3 days Sodium retention was greater and more consistent in the attacks induced by glucose and insulin (Fig 27 6) 2 methyl $9\alpha$ fluorohydrocortisone (Fig 27 7) DCA ammonium glycyrrhizinate and ACTH [25] than in the spontaneous attacks Unlike the reduction in potassium excretion which coincided with or followed the onset of

hypokalemia and paralysis the sodium retention preceded the paralytic spells and is therefore likely to be of greater significance in the pathogenesis of attacks [24] A negative sodium balance usually occurred for 1 to 2 days after the end of the attacks and was sometimes of considerable magnitude Sodium retention during attacks has been reported also by Pudenz et al [95] and Ferrebee et al [18] and has been confirmed more recently by Eales et al [120] and de Graeff and Brocker [132] An increase in exchangeable body sodium has been demonstrated to result from the sodium retention which preceded an attack of paralysis induced by $2\alpha$ methyl $9\alpha$ fluorohydrocortisone [24]

**Serum Sodium Concentration** The serum sodium tended to rise shortly before or during most seizures of severe paralysis or both in the three patients studied by Conn Streeten et al [117] This change was probably of little consequence in affecting muscle power but might have stimulated the thirst and oliguria which have frequently been commented on during attacks

**Effects of Restriction of Sodium Intake** Remarkable protection from spontaneous and induced attacks was found to result from restriction of the daily intake of sodium to 10 mEq or less combined with initial desalting procedures (using diuretics such as chlorothiazide mercurials and acetazoleamide) and quantitative replacement of the potassium losses resulting from the diuretics used [23–25] Thus in contrast with the frequent spontaneous attacks on a normal sodium intake of 176 to 190 mEq per day shown in Fig 27 5 there was only one bout of 3+ paralysis on the fourth day of a 23-day period on a 10 mEq sodium diet which was not combined with desalting No attacks occurred in over 6 weeks on an 8 mEq sodium diet after initial administration of acetazoleamide and mercaptomerin During the latter period of 44 days two separate attempts to induce paralysis with 2-day courses of glucose and insulin administration and one attempt with $2\alpha$ methyl $9\alpha$ fluorohydrocortisone (see Fig 4 in Conn et al [23]) were quite unsuccessful whereas previously and subsequently these stimuli never failed to induce paralysis in the same subject Similar results [25] have been obtained in another patient who was readily paralyzed on the second day of receiving repeated doses of glucose (150 gm) and insulin (20 to 35 units) on a normal sodium intake (200 mEq per day) On an intake of 6 mEq per day however even without preliminary desalting this patient was protected from the paralytic effect of the same course of glucose and insulin administration These observations on the protection afforded by a low sodium intake have been completely confirmed in two other patients studied by the authors including one with thyrotoxicosis [25] in the first of two studies by Eales et al [120] and in the less severe of two patients reported in brief by de Graeff and Brocker [132] It is not clear whether the incomplete protection afforded by a low sodium diet in the other studies

of Eales and de Graeff et al might have been attributable to inadequate sodium depletion or to failure to replace the potassium lost by the action of the diuretics used

*Chloride*

Changes in the urinary chloride excretion followed closely the changes in the urinary output of sodium There was reduced urinary excretion before and during attacks and diuresis at the end of the attack [18, 23 69] Like the serum sodium the serum chloride concentration tended to rise before and during attacks and this change was associated with approximately reciprocal changes in $CO_2$ combining power [18, 25]

*Water*

**Total Body Water** In many of the severe spontaneous and induced attacks observed in three patients on a constant diet [25] the sodium retention which usually preceded paralysis was associated with gain in weight signifying retention of water An increase in body weight before attacks was observed previously by Neel [90] who attributed the gain in weight to the fall in urine volume which he and others [69] had observed On the other hand Ferrebee et al [18] Talbott [49] and Danowski et al [119] found negligible changes in body weight before attacks The weight gain in the patient studied for the longest period of time by Conn et al [23–25] was greatest before the attacks which followed administration of the potent salt retaining substances DCA (3 2 kg) and ammonium glycyrrhizinate (2 2 kg) It was smaller before attacks which occurred after 2$\alpha$ methyl 9$\alpha$ fluorohydrocortisone (1 kg) glucose and insulin (1 1 kg and less than 0 5 kg) and spontaneously (0 3 0 8 0 2 −0 2 0 1 −0 3 −0 5 kg) Poor correlation between the magnitude of weight change and the severity of the attacks suggested that an increase in total body water was of little or no importance in the genesis of the paralytic seizures Support for this suggestion was derived from the finding that no paralysis resulted from the administration of pitressin tannate in oil despite an increase in the body weight of 3 2 kg in 3 days (see [25]) It may have been important that the severe retention of water induced by the pitressin tannate was associated with a negative sodium balance of 133 mEq (excluding fecal losses) This natriuresis was similar to that which follows the administration of pitressin to normal subjects, in whom it has been shown to be associated with reduction in aldosterone output [133 135] There are a few reports of paralytic seizures which occurred after the administration of water [17 136] but other authors [89 103 137] have failed to confirm that water loads even as large as 4 2 liters would precipitate an attack

**Changes in Fluid "Compartments"** Ferrebee et al [18] and Danowski et al [119] found that paralytic attacks were associated with no signif

icant change in extracellular fluid volume Danowski et al [119] observed a rise of 17 per cent in the plasma volume during attacks however Talbott [49] found plasma and extracellular fluid volumes to be increased in his patient as much as during attacks of paralysis In a single determination by Streeten and Conn [75] the bromide space between attacks was greater than normal Whether this implied an increased extracellular fluid volume or abnormal permeability to chloride of the tissues (muscle for example) which are usually relatively impermeable to this ion was not determined McArdle and Merton [130] found that the cellular water content was decreased to 21 4 liters in a patient with periodic paralysis between attacks compared with a normal value of 30 5 liters

Inadequacy of the available evidence makes it difficult to be certain of real changes in the internal distribution of body water in periodic paralysis although the published results suggest an expansion of plasma volume with conflicting evidence bearing on changes in the extracellular and intracellular compartments If such changes were aggravated before attacks one would expect them to result in a fall in the hematocrit In studies of three attacks it was found by Ferrebee et al [18] that the hematocrit remained unchanged early in the attacks but showed a downward trend during attacks from a mean value of 42 6 per cent at the onset to 39 7 per cent at the end of the attacks Since these changes did not precede the paralysis and since the downward trend in hematocrit during paralysis continued until 24 hr after complete recovery in the two patients in whom it was recorded these observations suggest that the development of paralysis was not associated with any significant internal shift of body water

CHANGES IN ADRENOCORTICAL FUNCTION

*Steroid Excretion*

Apart from reports of occasional 17 ketosteroid values [108 114] no systematic studies of steroid excretion had been reported in patients with periodic paralysis prior to the work of Conn Streeten et al [25] The urinary excretion of 17 hydroxycorticoids [138] 17 ketosteroids [139] and aldosterone (estimated by bioassay as described by Streeten et al [140]) is correlated in Fig 27 5 with the changes in serum potassium concentration and urinary sodium output in a number of spontaneous attacks observed in one patient In Fig 27 6 a similar correlation is shown in four attacks induced by glucose and insulin in the same patient and in two others with sporadic periodic paralysis The aldosterone values shown in Fig 27 6 were obtained using the chromatographic method of Neher and Wettstein [140a] as modified by Louis [122]

**Urinary 17 Hydroxycorticoids** A definite increase in the output of 17 hydroxycorticoids occurred on the first and second days of paralysis

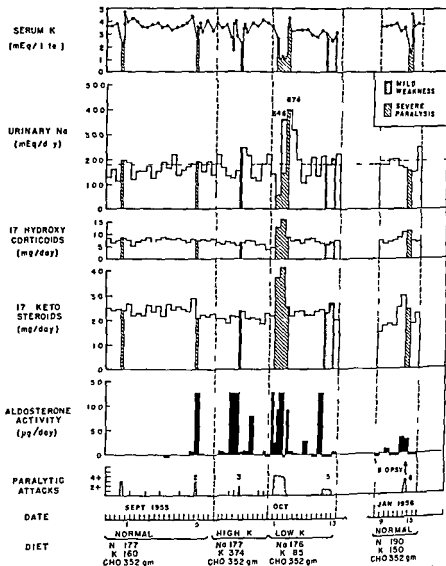



Fig 27-5 Serum potassium and sodium and steroid excretion in patients with spontaneous attacks of periodic paralysis

In this and subsequent figures illustrating metabolic data *severity of paralysis* is indicated as follows 4+ = complete quadriplegia 3+ = severe paralysis of the limbs 2+ = weakness of arms and legs 1+ = mild weakness of arms or legs

Electrolyte (e g sodium) intake is indicated by the dotted line and urinary output is plotted from the base line upwards so that when urinary output exceeds intake the output column rises above the dotted line depicting intake

The cross hatched areas (Nos 1 to 6) indicate the duration of major attacks of paralysis

in the most severe spontaneous attack (Fig 27 5 No 4) and on the day before and the day of the next most severe spontaneous attack (No 6) An increase in 17 hydroxycorticoids was also seen in the two severe attacks (Fig 27 6 Nos 7 8) induced by glucose and insulin The 17 hydroxycorticoid output was higher on the day of each of these attacks than on any other day of the study The mild attacks both spontaneous (Fig 27 5) and induced (Fig 27 6 No 9) were accompanied by no demonstrable change in 17 hydroxycorticoid excretion

**Urinary 17 Ketosteroids** The 17 ketosteroids showed an undoubted increase in the two severe attacks [*25*] (Fig 27 5 Nos 4 6) Moreover even the less severe attacks were always associated with an increased output of 17 ketosteroids on the day of the attack (Nos 3 5) or the day immediately before (No 2) or after (No 1) the attack Again the fact that the 17 ketosteroid output at the time of the attack was in every instance the highest excretion reported for 2 to 14 days before and after each attack suggests that these changes are significant despite the relatively wide day to day fluctuation in 17 ketosteroids in this patient The increase in excretion of 17 ketosteroids *before* attacks (Nos 2 6) suggests that adrenal hyperactivity or whatever change in steroid metabolism caused this augmentation of 17 ketosteroid output might have been concerned in the causation and was certainly not the result of the paralytic attacks This interpretation is strengthened by the observation that a severe attack of complete paralysis which occurred after the administration of the potent nonsteroidal sodium retainer ammonium glycyrrhizinate (a triterpene) was associated with no significant change in 17 ketosteroid output Thus the 17 ketosteroids were 20 6 18 9 and 18 1 mg before administration of the triterpene 20 0 and 18 4 mg on the first and third days of its administration 17 1 and 19 1 mg on the 2 days of the attack and 17 0 mg on the following day The evidence suggests therefore that the increased output of 17 ketosteroids before or during attacks of paralysis is a characteristic feature of the metabolic events leading to spontaneous seizures but one which plays no essential role per se in inducing paralysis Cerny et al [*114*] in the protocol of one of their patients with familial periodic paralysis recorded an increase in the urinary 17 ketosteroids from 5 8 mg per day between attacks to 17 3 mg per day on the day of an attack This observation is in agreement with those described above

**Urinary Aldosterone Output.** The urinary excretion of aldosterone-like material was estimated by bioassay before during and after five of the spontaneous attacks (Fig 27 5 Nos 2 to 6) In every instance a large increase in excretion of aldosterone like substance occurred on the day before and usually also on the day or days of paralysis Excretion of salt retaining steroid was normal (less than 3 $\mu g$ per day) on several of the days between attacks but was not infrequently increased on two days

between attacks when a great increase in aldosterone output occurred sharp sodium retention also occurred (Sept 26 and Oct 7, 1955) In addition one of these days (Oct 7) was followed by the only reduction in serum potassium concentration below 4 mEq per liter observed among

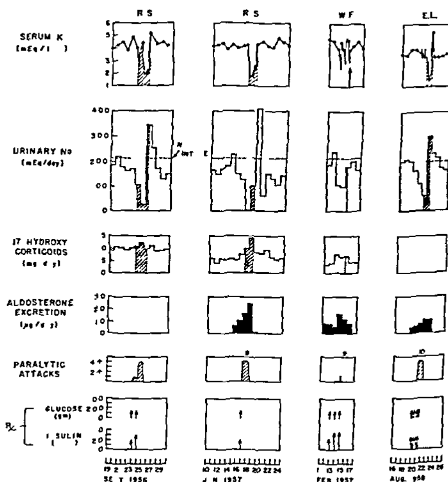



Fig 27-6 Serum potassium and urinary sodium and steroid excretion in attacks of periodic paralysis induced by glucose and insulin Symbols as in the legend for Fig 27-5

52 determinations obtained between attacks in this patient These findings may therefore have represented an 'abortive spontaneous attack.

The attacks induced by glucose and insulin in the three patients (Fig 27-6) were also associated with increased urinary output of aldosterone (measured chromatographically in these studies) The more

modest rise in aldosterone output in these induced attacks might simply reflect the differences in technique or might indicate that the spontaneous attacks were really associated with far greater adrenocortical hypersecretion than occurred after glucose and insulin Bearing in mind the obvious limitations of the bioassay [140 141] one is probably justified in attributing this difference to technical factors until further evidence to the contrary has been adduced Eales [136] has confirmed the finding of an elevated aldosterone output during two spontaneous attacks

Since the increased output of adrenocorticoids in the urine before and during attacks involved three different steroids and since paralytic attacks were associated with no obvious changes in hepatic or renal function it seems reasonable to conclude that increased adrenocortical secretion was the cause of the increased urinary excretion of these metabolites This conclusion receives support from the evidence of the biologic effects of increased adrenocorticoid output

*Biologic Effects of Increased Adrenocortical Steroid Output*

**Electrolyte Metabolism** Since the augmented output of aldosterone correlated reasonably well with the sodium retention and the decreases of serum potassium (Fig 27 5) there is reason to believe that hypersecretion of aldosterone might have been the cause of these electrolyte effects The rise in serum sodium associated with the attacks [23 24] was probably also attributable to hypersecretion of aldosterone The urinary output of potassium was reduced (Fig 27-4), rather than increased as would have been expected normally during greatly increased aldosterone secretion Thus a fundamental defect in periodic paralysis (unavailability of potassium for urinary excretion during attacks) is again evident It is significant that administration of mineralocorticoids to such patients results in the same abnormal pattern of renal excretion of electrolytes

**Organic Metabolism** Changes in protein metabolism during paralytic attacks have been reported by Conn Streeten et al [25] Figure 27 7 shows the urinary nitrogen output before during and after 14 attacks of paralysis in one patient (R S ) the duration of attacks is indicated by crosshatching Fecal losses of nitrogen were not estimated except in the first attack (Oct 1 1955) in which the occurrence of a slightly negative nitrogen balance on the first and third days of the attack is shown Urinary losses of nitrogen were so great on the day of or after each of the spontaneous attacks or both however that they frequently exceeded total nitrogen intake The attacks induced by glucose and insulin (Nos 10 11) and by 2α methyl 9α fluorohydrocortisone (No 12) were also associated with a negative nitrogen balance but no increase in urinary nitrogen output occurred in the attacks induced by DCA (No 13) and ammonium glycyrrhizinate (No 14) The steroid 2α methyl 9α fluoro-

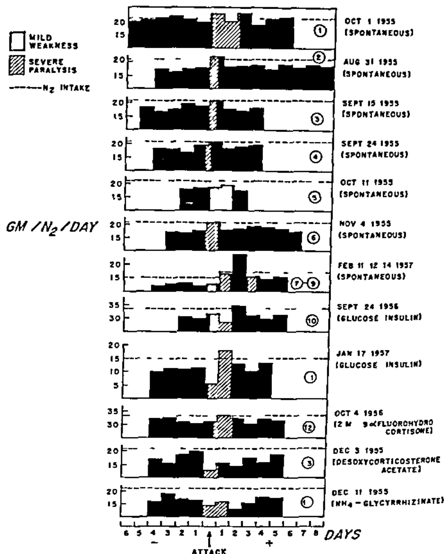



Fig 27 7 Changes in urinary nitrogen in a patient during 14 attacks of periodic paralysis The data are plotted as in Fig 2 5

hydrocortisone has sufficient glucocorticoid potency to increase urinary nitrogen excretion when given to normal subjects in doses as low as 0 5 mg by mouth [142] The increased nitrogen excretion observed in the patient with periodic paralysis given twice this dose (Fig 27 7 No 12) therefore might well have resulted from the glucocorticoid activity of the steroid administered and does not imply an increased endogenous release of glucocorticoids DCA and ammonium glycyrrhizinate are both known to possess potent mineralocorticoid activity and to be practically

devoid of glucocorticoid activity The absence of increased nitrogen excretion in the severe attacks which followed their administration therefore suggests that the excessive adrenocortical secretion of a gluco corticoid (probably hydrocortisone) in the spontaneous attacks neither played an essential role in the genesis of muscular paralysis nor resulted merely from the stress of the seizures

Uric acid excretion was increased from a mean control level of 0 93 gm per day to 1 05 to 1 54 gm per day on at least one of the days in each of six spontaneous paralytic attacks studied in the same patient [25] On the only day between attacks when the urinary uric acid excretion exceeded 1 05 gm per day there were also sodium retention a fall in serum potassium to 3 9 mEq per liter and increased 17 hydroxycorticoid excretion with increased aldosterone output on the preceding day suggesting that an abortive attack was taking place The increased uric acid excretion presumably reflected the effects of increased adrenocortical secretion of hydrocortisone

**Other Effects of Adrenocortical Hyperactivity** Urinary ammonia was greatly increased on the day of paralysis in four spontaneous attacks and in one attack which occurred probably spontaneously on the second day of prednisolone therapy (following spontaneous increase in aldosterone secretion the day before the attack) The same phenomenon occurred during attacks induced by an upper respiratory infection and by administration of glucose and insulin $2\alpha$ methyl $9\alpha$ fluorohydro cortisone DCA and ammonium glycyrrhizinate [25] Increased urinary ammonia probably resulted from increased mineralocorticoid activity since it was seen in the attacks which followed administration of DCA and ammonium glycyrrhizinate and is known to occur in primary aldosteronism where there is no increase in 17 hydroxycorticoid excretion [122] The resemblance of this increased urinary excretion of ammonia to that which occurs under the influence of excessive aldosterone secretion was further manifested by the striking phenomenon of its persistence through the second day of three severe attacks of paralysis when urinary pH was increased above 7 0

*Urinary pepsinogen excretion* increased on the day of or after each of three spontaneous attacks one attack induced by ACTH gel one which followed an upper respiratory infection two induced by glucose and insulin and one precipitated by $2\alpha$ methyl $9\alpha$ fluorohydrocortisone [25] A rise in the urinary excretion of pepsinogen may result from increased renal clearance of pepsinogen in response both to adrenocortical stimulation [143] and to infusion of hydrocortisone [144] It has been encountered in primary aldosteronism [144a] Increased urinary pepsinogen excretion in periodic paralysis therefore presumably results from the increased adrenocortical secretion of hydrocortisone or of aldosterone or both

### SIGNIFICANCE OF THE INTERMITTENT ADRENOCORTICAL HYPERSECRETION

It has been shown that both spontaneous attacks and attacks induced by glucose and insulin are accompanied by a transiently increased secretion of adrenocortical steroids This phenomenon which frequently starts before paralysis supervenes constitutes a characteristic feature of the paroxysms and is not merely the consequence of the "stress" of paralysis (see discussion under Organic Metabolism above)

*Elevation of Urinary 17 Hydroxycorticoids*

This probably results from increased secretion of hydrocortisone since the usual results of such an increase are readily recognizable negative nitrogen balance [25] (see under Organic Metabolism above) greater excretion of uric acid [25] mildly elevated FBS [10 25, 47 56 114] leukocytosis [8] lymphopenia [92] and eosinopenia [8] These changes are quite mild, however The administration of glucocorticoids in amounts equivalent to or even greater than the adrenocortical output of hydrocortisone which would be estimated to occur before or during these attacks (probably never more than twice the normal adrenocortical output of 25 to 35 mg hydrocortisone daily) does not cause paralysis [22] For these reasons and because severe paralysis may result from procedures which do not increase glucocorticoid activity in the body (e g administration of DCA and ammonium glycyrrhizinate) there is little doubt that excessive secretion of hydrocortisone plays no essential role in the genesis of paralysis

*Increased Output of Urinary 17-Ketosteroids*

This increase is more consistent than the augmentation of 17 hydroxycorticoid excretion It may result from increased adrenocortical production of adrenal $C_{19}$ steroids from increased hepatic formation of 11 oxygenated 17 ketosteroids from glucocorticoids or from increased testicular secretion of androgens In the absence of further evidence which might be obtained by chromatographic fractionation of the elevated urinary 17 ketosteroids it is difficult to interpret the significance of this change *There can be little doubt that while this change too is an* integral part of the metabolic events inherent in the genesis of paralysis it plays no direct role in paralyzing the muscles

*Increased Output of Aldosterone*

This is the steroid change which most consistently precedes and accompanies spontaneous paralytic seizures Furthermore an increase in the salt retaining aldosterone-like activity in the body is the only known factor which is common to almost all if not all of the means by which

paralysis may be induced emotional upsets [145] infections surgical and other physical trauma [146] increased rate of carbohydrate utilization resulting from glucose and insulin administration (see Urinary Aldosterone Output above) and the administration of ACTH [147 148] adrenocortical extract DCA 2α methyl 9α fluorohydrocortisone and ammonium glycyrrhizinate There are therefore strong reasons for believing that a transient rapid increase in aldosterone production plays an important role in the pathogenesis of paralysis

This evidence is strengthened by the observation in four patients [24 25] that the paralytic effects of glucose and insulin were completely abolished by feeding a low salt diet Most if not all of the metabolic effects of aldosterone are known to be abolished by sodium restriction This procedure may ameliorate or entirely reverse many of the abnormal features of severe primary aldosteronism [149] Additional confirmation of the pathogenic role of transient hyperaldosteronism in precipitating attacks has been adduced by the administration of a spirolactone (SC8109) to these patients Adequate doses (500 mg q 6 h p o ) of this drug which is known to antagonize the *effects* of liberated aldosterone [150 151 152] prevented the sodium retention which was otherwise always associated with the administration of glucose and insulin to one patient In another patient with severe periodic paralysis associated with thyrotoxicosis the same spirolactone protected the individual from paralysis during 3 days of glucose and insulin administration although 1 or 2 days of the latter treatment had previously paralyzed him consistently [25]

## NATURE OF THE RELATIONSHIP BETWEEN TRANSIENT HYPERALDOSTERONISM AND PARALYSIS

Consideration of the evidence bearing on the following questions may serve to clarify the nature of the defect in periodic paralysis

1 *Is the hyperaldosteronism due to an excessive stimulus to aldosterone production or to an excessive response by the adrenal gland to a normal stimulus?* Excessive secretion of aldosterone has been observed repeatedly in spontaneous attacks which were preceded by no known psychic or physical trauma and by no acute dietary excess of carbohydrate (the patient being on a constant diet in the sheltered environment of the metabolic ward) These observations imply either that the adrenal gland was unduly sensitive to normal day to-day stimuli or that some defect in the hypothalamus or elsewhere was resulting in intermittent excessive release of glomerulotropin [153] If the former alternative were correct it would be anticipated that the release of aldosterone would have been greater in these patients than in normal subjects following the same stimulus Administration of glucose and insulin to normal young adult males has revealed increases of 17 hydroxycorticoids 17 ketosteroids and

*aldosterone which were very similar to the changes seen in the patients* with periodic paralysis [122] Thus it appears that the increased carbohydrate utilization which results from the administration of glucose and insulin is a stimulus to adrenocortical hypersecretion in normal subjects In addition, Conn et al [117] have found that the increase in urinary aldosterone resulting from sharp restriction of dietary sodium in normal people is seen also in patients with periodic paralysis Thus the adrenal glands of patients with periodic paralysis probably possess no *inherent* abnormality in their responsiveness to physiologic stimuli The implication is that intermittent hyperaldosteronism in periodic paralysis arises from an extraadrenal abnormality which stimulates hypothalamic release of glomerulotropin or from a primary defect in the hypothalmus characterized by intermittent "spikes of glomerulotropin release

It may be of some interest that glucose (150 gm) and insulin (20 units) in amounts which induced quadriplegia in a patient with severe periodic *paralysis have been administered to a patient with severe primary aldosteronism* due to an adrenocortical adenoma [70] No muscular weakness ensued Aldosterone excretion did not increase but instead, fell from 39 to 30 $\mu$g per day and the preexisting sodium retention was not aggravated in this patient in response to glucose and insulin These findings indicate that the aldosteroma which proved to be present in one adrenal gland was not responsive to the physiologic stimuli to which normal adrenal glands respond Certainly the responsiveness of the adrenal glands in patients with periodic paralysis to glucose and insulin administration and to other physiologic stimuli appears to be normal On this basis as well as upon the basis of what scanty autopsy evidence is available one can eliminate with reasonable certainty, the likelihood that adreno cortical tumors are involved in the pathogenesis of periodic paralysis The conclusion seems justified that in this disease there occurs periodically an excessive stimulus which results in the release of abnormally large amounts of aldosterone and that this stimulus arises by virtue of an abnormality which is not localized in the adrenal gland

2 *Is there a primary defect in muscle membrane permeability?* Evidence can be marshaled for and against this proposal Patients with periodic paralysis are regularly paralyzed by doses of 2$\alpha$ methyl 9$\alpha$ fluoro hydrocortisone (1 mg given once p o ) or deoxycorticosterone acetate (12 5 mg b i d intramuscularly) Such quantities of mineralocorticoids cause smaller decreases of serum potassium and no paralysis when administered to normal *subjects Figure 27 8 shows that a single dose of* 2$\alpha$ methyl 9$\alpha$ fluorohydrocortisone (1 mg p o ) produced a far greater fall in serum potassium in two patients than in two normal male subjects of comparable age and on the same diet This suggests that an abnormality of membrane permeability to potassium is present in periodic

paralysis With respect to sodium however there is no evidence for the presence of a membrane defect in the kidney since the degree of sodium retention following this dose of 2α methyl 9α fluorohydrocortisone was not abnormal either at the time of onset of paralysis in the two patients

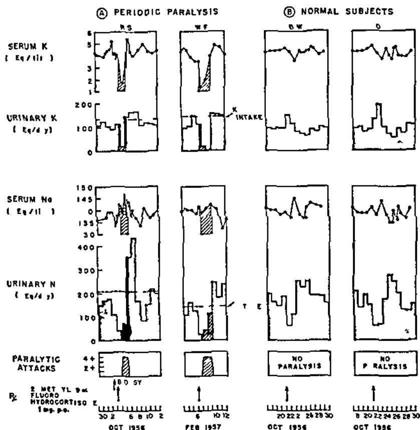



Fig 27 8 Effects of 2α methyl 9α fluorohydrocortisone on serum concentration and urinary excretion of sodium in normal subjects and in patients with periodic paralysis

or over the subsequent 3 days when compared with the changes in normal subjects The paradoxic fall in urinary potassium excretion in the patients does not necessarily indicate the presence of a membrane defect since it might be explained by the more rapid and profound reduction of serum potassium as the result of more rapid sequestration of potassium presumably in the muscles in these individuals than in normal subjects (see Changes in Urinary Potassium above)

Immersion of one arm in cold water for 20 to 35 min may cause severe

weakness of the muscles of that arm [*41, 59, 71, 90*] without a concomitant fall in the serum potassium concentration in the weakened limb [*41, 59*] This observation led McArdle [*41*] to conclude that the muscles them selves were abnormal in periodic paralysis and to postulate that the defect lay in the muscle membrane permeability The abnormalities in muscle electrolyte composition (see under Chemical Composition of Skeletal Muscle, above) and other evidence confirm the conclusion that the muscle itself is abnormal but they do not eliminate the possibility that this abnormality might be secondary to a remote cause, such as a prior, chronic excess of aldosterone reaching the muscle through the circulating blood It is quite possible, too that cooling might inhibit the association of actomyosin in the muscles of the patient as it has been shown to do in vitro [*126*] In the presence of abnormal electrolyte com position of the muscle this inhibitory effect of cooling might be sufficient to weaken or paralyze the muscle Whatever the exact nature of the local effect of cold on muscle function is it might be significant that exposure to cold has precipitated paralysis in patients with primary aldosteronism [*154*] in whom composition of the muscles is deranged in the same way as it is in periodic paralysis (Table 27 3) The finding *that in periodic paralysis the influx of potassium into muscle is not* exaggerated but normal between attacks [*20*] may be cited as further evidence against any intrinsic defect of the muscle membrane

On the other hand one could postulate a possible sequence of events based upon a primary abnormality with respect to potassium in bone *muscle, or both in which (1) sequestration of potassium slowly increases* to a critical value (2) this excites release of aldosterone, (3) the latter in the presence of adequate sodium produces a simultaneous increase in intracellular sodium and (4) abnormal muscle function or paralysis re sults from excessive intracellular concentration of cations

## OTHER HORMONAL CHANGES

### *Follicle Stimulating Hormone (FSH)*

Cerny et al [*114*] have reported in a patient with familial periodic paralysis that urinary excretion of FSH was greatly increased to 80 and 400 rat units a day during two attacks and fell to below 20 rat units a day on two days between attacks The low level between attacks was confirmed in two other patients

### *Thyroid*

Thyroid function has been normal in patients with *sporadic periodic* paralysis who had no clinical evidence of thyrotoxicosis [*75*] In those patients who have had the clinical features of thyrotoxicosis associated with periodic paralysis there has been adequate laboratory evidence of hyperthyroidism in the form of elevated BMR, increased serum PBI

concentration and increased 1 2, and 24 hr uptake of $I^{131}$ by the thyroid gland [53 55 94 116, 155] The muscular disorder in these patients is similar to that found in the more usual euthyroid patients in that attacks are predominantly nocturnal show a predilection for resting muscles are always associated with a profound fall in serum potassium level and can be prevented by a low sodium diet as well as by administration of the aldosterone antagonist SC8109 [155] These observations and the findings that attacks of paralysis in patients with thyrotoxicosis are associated with an increase in aldosterone output [156] suggest that increased secretion of thyroid hormone may induce adrenocortical release of aldosterone This possibility, the existence of which has yet to be demonstrated conclusively would be consistent with the report of Shinosaki [10] that bouts of paralysis occurred with greater frequency in patients with periodic paralysis who were treated with large do es of desiccated thyroid Since an increased output of ACTH glomerulotropin and FSH has been demonstrated or inferred before or during attacks it might be speculated too that an excess of yet another tropic hormone thyroid stimulating hormone might be responsible for the thyrotoxicosis in these patients There is at present no direct evidence to support this possibility

## PATHOGENESIS

The available evidence is insufficient to establish conclusively the nature of the primary defect in periodic paralysis However the hypothesis to be advanced here is consistent with what evidence there is and might be useful in indicating where future studies would be expected to be fruitful

Measurement of adrenal steroid excretion during spontaneous and induced attacks of paralysis has provided indirect and admittedly tenuous evidence that the primary defect in periodic paralysis might consist in the occurrence of intermittent excessive stimuli to the secretion of adrenocorticoids particularly aldosterone The possibility that these stimuli arise from the hypothalamus would be supported by the reported occurrence of periodic paralysis in patients with concomitant manifestations of hypothalamic disorders [51 113 157]

Intermittent mildly excessive secretion of aldosterone over a period of many years between attacks probably produces a slightly negative pota sium balance by increasing urinary losses of potassium as McArdle [41] has observed at least temporarily in a patient who ingested a diet containing low normal amounts of potassium This like all other losses of potassium from the body would be expected to cause depletion of potas ium in the skeletal muscle and its replacement by sodium The e shifts would produce the observed abnormalities in muscular electrolyte composition as well as in exchangeable body sodium and potassium

between attacks for which no other explanation is at present available In this way chronic low grade more or less intermittent excesses of aldosterone secretion probably set the stage in the skeletal muscle (and perhaps in the myocardium and even the smooth muscle as well) for the more severe sudden increases in aldosterone secretion which result from physical or psychic trauma ingestion of unusual amounts of carbohydrate foods, or perhaps 'spontaneous' hypothalamic discharge of glomerulotropin Such sudden marked increases in the secretion of aldosterone as well as of hydrocortisone and other adrenocorticoids occur immediately before attacks of paralysis The effects of these increased rates of steroid secretion may be recognized in the negative nitrogen balance increased uric acid excretion eosinopenia leukocytosis sodium retention and increased urinary excretion of pepsinogen and ammonia none of which probably plays any direct role in the pathogenesis of paralytic attacks That paralytic attacks occur mainly at night and in resting muscles may result from the additive effects of rest and an excess of circulating aldosterone both promoting the rapid uptake of potassium by the muscles of which there is strong evidence during the attacks from a number of sources [*19 20 21 25, 96, 108*] That aldosterone does have a direct effect on skeletal muscle increasing its potassium content and reducing its sodium has recently been shown in acute experiments on mice [*158*] which await confirmation The contrary opinion that aldosterone decreases muscle potassium and increases muscle sodium has been based largely upon indirect evidence from the effects of chronic aldosterone excess where renal wastage of potassium was probably responsible for the potassium deficiency and sodium excess in the muscles

Under the influence of rest and acute hyperaldosteronism the influx of potassium into the muscle cells appears to be more rapid than the loss of sodium so that the muscles come to contain too large an amount and frequently too high a concentration of sodium plus potassium as has been shown in biopsy studies during paralysis (see under Chemical Composition of Skeletal Muscle above) It is probably the *increased amount of sodium + potassium* in the muscle cells which interferes with the association of actomyosin [*126 128*] and in this way causes paralysis Hyperpolarization of the muscle end plate and membrane resulting from the sudden rise in intracellular potassium concentration and the fall in serum potassium concentration might play a less important part in paralyzing the muscle The fall in serum potassium concentration and in urinary excretion of potassium during the attacks probably result from rapid uptake of potassium by the muscle

## DIFFERENTIAL DIAGNOSIS

When repeated attacks of flaccid muscular paralysis have occurred upon awakening in a young subject who appears to have been in perfect

health between attacks the only conditions which might be confused with periodic paralysis are adynamia episodica hereditaria and hysteria *Adynamia episodica hereditaria is readily differentiated by the clinical* differences enumerated in Chap 28 and by the absence of a fall in serum potassium during an attack Hysteria may be recognized by its characteristic psychologic features by the usual presence of sensory changes the persistence or even accentuation of reflexes the predilection for females and the retention of the muscular responses to electrical stimulation

Less severe attacks causing weakness only may present more difficult diagnostic problems The presence of muscular atrophy early in the course of the disease would favor a diagnosis of *progressive muscular atrophy* of spinal origin or one of the primary muscular dystrophies since in those few familial cases of periodic paralysis which manifest this sequela muscle wasting occurs only late in the course of the disorder [12] The weakness of *myasthenia gravis* is characteristically least in the mornings and increases during the day it involves facial and ocular muscles early frequently lacks the intermittency of periodic paralysis responds dramatically to the administration of neostigmine and is not associated with hypokalemia though it may be improved by the administration of KCl The myotonic components of *dystrophia myotonica* and *myotonia congenita* are absent in periodic paralysis *Peripheral neuritis* produces sensory as well as motor impairment

In a first attack of paralysis several other possibilities have to be excluded such as *cerebral lesions* (vascular accidents tumor etc ) *spinal lesions* (tumor syphilis poliomyelitis) and *lesions of peripheral nerves* (polyneuritis Landry s paralysis Guillain Barré syndrome and diabetic neuropathy) The cerebrospinal fluid and concomitant neurologic abnormalities will usually enable these conditions to be differentiated from periodic paralysis without difficulty

*Primary aldosteronism* even when associated with repeated attacks *of paralysis is* distinguished from periodic paralysis by the occurrence of hypertension and the persistence of hypokalemia between attacks

## MORTALITY AND PATHOLOGIC FINDINGS

Talbott [49] has drawn attention to the fact that in about 10 per cent of the patients reported in the literature death has occurred during attacks of paralysis A fatal outcome has been seen in sporadic as well as in hereditary cases and a particularly high rate of mortality has been recorded in some families such as Holtzapple s [60] in which six patients died in attacks of paralysis (Fig 27 2) The causes of death when related to the disease have been mainly *respiratory paralysis [56 57 60 115]* respiratory infections [46 60] inhalation pneumonia [10] cardiac failure [71] shock [51] and excessive therapeutic venesections [10 46 79]

*Autopsies* have been reported on a number of patients who died in or

out of attacks [7 10 16 46, 57, 60 79 90 114, 159] The changes of Graves disease were seen in the thyroid of a patient who had hyper thyroidism [10] and evidence of cardiomegaly and venous congestion in patients who died in cardiac failure [114] Shinosaki [10] considered that the histologic appearance of the parathyroids was compatible with hyper function of these glands Unless this is a form of compensatory (secondary) hyperfunction it would be difficult to reconcile the invariable absence of hypercalcemia during attacks with primary hyperparathyroidism al though the latter could explain the fall in serum phosphorus which usually occurs [13] The adrenal and other endocrine glands are not reported to have shown gross abnormalities Whether histologic studies were routinely performed on these glands in the studies reported is not stated

The only pathologic changes which appear to be specific for periodic paralysis are found in the skeletal muscles Goldflam [8] was the first to report the occurrence of histologic changes in the muscles in two brothers with periodic paralysis The lesion comprised an increase in the diameter of the individual muscle fibers rarefaction of the primitive fibrils and the formation of numerous vacuoles within individual muscle fibers The appearance of hypertrophied fibers in the muscles of patients with periodic paralysis has been confirmed by Schmidt [46] and Crafts [66] The more striking picture of vacuoles within the muscle fibers has at tracted the attention of numerous authors who have debated whether these findings were a manifestation of real pathologic change or merely artifactual Goldflam [8] himself considered that the changes bore a causal relationship to the paralytic seizures and used their presence to exclude the possibilities that the paralysis resulted from neurosis or from a lesion of the central nervous system In agreement with Goldflam [8] Singer and Goodbody [14] were convinced that the vacuolar changes were not seen in sections of normal muscle and believed that the ex traordinary fissuring seen on transverse sections of skeletal muscle was the result of some essential abnormality in the muscle fibres' Schmidt [46] found that some of the vacuoles were filled with granular masses which stained like glycogen with Best s carmine stain while others were filled with a homogeneous transparent shiny substance which did not take up the stains used He was emphatic that these changes were not seen in muscle from control subjects Neel [90] appar ently unwilling to accept the verdict of his pathologist that the changes were artifacts pointed out that similar findings were absent from the muscles of his patient s unaffected sister as well as from the muscle of three patients of similar age who did not have periodic paralysis Zabriskie et al [71] confirmed the findings of Schmidt [46] that some of the vacuoles were filled with glycogen granules and shared the view that these lesions were indicative of pathologic change in the muscles Biemond and Daniels

[12] and Allott and McArdle [16] also observed the vacuoles but the former authors dismissed them as artifacts

Conn et al [23 24] showed by serial biopsies on a patient with sporadic periodic paralysis that the vacuoles were easily recognizable when the patient was paralyzed by glucose and insulin on a high sodium intake (Fig 27 9A) but disappeared completely (on biopsy from the same muscle) at a time when sodium depletion and a low sodium intake had reduced the sodium content of the muscle (see Table 27 2) as shown in Fig 27 9B Specific stains failed to demonstrate the presence of either

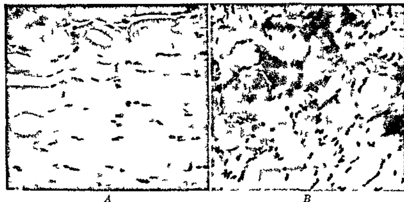



Fig 27 9 Photomicrographs of skeletal muscle from Patient R S showing vacuoles within fibers of muscle paralyzed on high-sodium diet (A) and disappearance of these vacuoles on low-sodium diet when paralysis could not be induced (B)

glycogen or lipid material in the vacuoles These findings suggest that the vacuoles contained mainly sodium chloride and water and that the accumulation of excessive amounts of sodium and water within the muscle fibers might constitute an important step in the pathogenesis of periodic paralysis

## GENETICS

From the numerous published pedigrees of patients with familial periodic paralysis it is evident that in most instances the defect is inherited as an autosomal dominant trait with complete penetrance [12 45 47 56 57 59 62 86 93 114 160 161] From three of the larger kindreds collected from the literature and shown in Fig 27 3 it can readily be seen that one of the parents of each affected individual invariably suffered from the malady A few authors have published pedigrees which indicate dominant autosomal inheritance with incomplete penetrance [40 44 46 60 89] Three of these are illustrated in Fig 27 2

It is not always clear whether the authors who described incomplete penetrance were able to verify the alleged absence of attacks in the parents of affected individuals by personal interviews with these parents

At least in some instances evidence of incomplete penetrance is inconclusive, since it appears to be based upon the testimony of relatives whose contact with the allegedly unaffected individuals might well have been tenuous A few patients have experienced their first attacks of paralysis in the fourth decade of life [10 12 93] or even at the age of 56 [9 61] and many authors [7 12, 47 56 90] have described members of affected families who in an entire life span experienced only a single attack or a few mild attacks all occurring within a brief period of time It is likely, therefore as Dalinghaus [48] and Oliver et al [93] have suggested, that variability in the age of onset and in the severity and frequency of the paralytic seizures may explain why the disease appears to have skipped a generation in some families If this suggestion is correct it might be expected that penetrance would appear to be less complete among females than among males since females are generally considered to suffer less severe and less frequent attacks than males [56 63] That females do predominate in the apparently skipped generations [40 46 89] lends support to this hypothesis The apparently normal parents of affected offspring might represent then *formes frustes* of the disease and might be expected to possess the fundamental underlying defect Nobody has yet attempted to induce paralysis is such allegedly unaffected parents of sufferers from the disease by the repeated administration of glucose and insulin or of salt retaining adrenocorticoids

One report has been published by Khan [98] describing the only Indian family in the literature in which the gene appears to have been sex linked and recessive The eight affected individuals in three generations were all males and in each case the disorder was inherited from an unaffected female No affected male transmitted the condition to his offspring The females in the pedigree were summarily dismissed with the statement that all the sisters of the listed males were unaffected Since the members of this family were Mohammedan peasants among whom the women usually refrain from social and even professional medical contact outside the family circle it is not unlikely that the author was unable to interrogate the females in person The importance of a reliable history from the females of this family is evident when one reflects that if only one of the women had been found to have suffered from paralytic episodes the pedigree would have lost its seemingly unique features, becoming very similar to those published by Sagild [40] Brisset [97] and others, with a strong predilection for males because of incomplete penetrance in females In the absence of more details this kindred cannot be considered to have established the existence of sex linked recessive inheritance in periodic paralysis

Talbott [49] has implied that the existence of skipped generations in some families with periodic paralysis indicates recessive inheritance in those families This implication is not generally considered valid as

Gates [162] has pointed out Similarly Brisset s [97] claim to have shown recessive inheritance in periodic paralysis cannot be accepted His claim rests entirely upon the finding of incomplete penetrance among the females in a pedigree where the occurrence of the malady in 8 of 17 adult descendants clearly indicates dominant inheritance

There appears therefore to be no conclusive evidence in the literature which is incompatible with the view that familial periodic paralysis is inherited as an autosomal dominant trait with complete penetrance in most families and with apparently incomplete penetrance in others

*Sporadic Cases of Periodic Paralysis*

Patients with no family history of paralytic episodes have attacks which are identical with the hereditary type in their clinical manifestations and in their biochemical changes [21 49 163] The age of onset is very similar (Fig 27 1) the same stimuli provoke attacks and thyrotoxicosis may bring to light the underlying metabolic defect by precipitating attacks in both groups of patients [10 98] While it is possible that inadequacy of information about the forebears may have been responsible for the failure to establish the truly hereditary nature of some allegedly sporadic cases a strong case has been made for the existence of truly sporadic instances of periodic paralysis by the diligent studies of Gaupp and Kalden [165] These authors traced and made personal contact with 186 out of the 261 living descendants of the grand parents of one of their patients and obtained adequate information from relatives and family physicians about another 50 of the descendants leaving only 17 per cent of the pedigree unaccounted for Of the 236 individuals about whom adequate information was available none had any history at all suggestive of periodic paralysis

Evidence for or against the existence of truly sporadic instances of periodic paralysis might be obtained by observing the responses of the siblings and parents of apparently sporadic patients to the administration of glucose and insulin or to adrenal salt retaining corticoids or simply to heavy meals followed by rest as Neel [90] suggested 30 years ago No fall in serum potassium concentration and no paralysis resulted from the administration of 4 mg 9α fluorohydrocortisone and 10 gm NaCl to the father and mother of a young man with sporadic periodic paralysis [75] While this result will obviously need to be confirmed on the relatives of other sporadic patients the consistency with which this stimulus will induce attacks of paralysis in affected patients supports the conclusion that the parents of this patient probably did not possess the genetic trait for periodic paralysis

Of the 92 apparently sporadic cases of periodic paralysis which the authors have been able to trace in the literature [6 10 14 16 19 21 23–25 31 50–56 64–69 76 78 82–85 87 88 91 92 94 98–103 105

*111, 116 118, 163-170*] only 7 have been females [*10 19, 21, 51, 84 88, 99*] The scarcity of female cases and the fact that their allegedly sporadic nature has sometimes been inconclusively documented [*10, 19, 88, 99*] or actually disproved by the transmission of the disorder to the offspring [*21*] allow the speculation that sporadic cases may not really occur at all in females It is possible that the apparently sporadic cases in males might result from inheritance of a recessive sex linked gene In the present state of ignorance however it cannot be stated with certainty whether sporadic instances of periodic paralysis result from recessive inheritance, whether they represent new mutations which will manifest dominant inheritance in succeeding generations, or whether their defect is acquired and not genetically determined in any way

## TREATMENT

The many forms of treatment that have been used in patients with periodic paralysis have included administration of Prostigmine, Mecholyl, carbaminoyl choline [*95*] desiccated thyroid [*32*] ovarian substance [*88*] and use of an oxygen tent [*116*] and milk diets with the content of carbohydrate not higher than 10 per cent [*137*] Avoidance of high carbohydrate intake, particularly in the evening appears to be a reasonable recommendation to the patient in view of the ease with which glucose loads precipitate attacks

Potassium salts were used empirically for many years before the detection of hypokalemia during the attacks [*14, 15 60*] The administration of large doses (5 to 15 gm) of potassium salts (usually KCl) appears, in the opinion of most observers to shorten the duration of paralysis considerably when given during an attack [*13 16 19, 21, 61, 89 95 114 115*] Improvement in muscle function may occur within 15 min or may be deferred for 2 hr or more and has been attributed to a reversal of the abnormally rapid influx of potassium into the tissues [*19*] Of the few authors who have failed consistently to observe beneficial effects from the therapeutic administration of potassium some have probably used too small doses [*15 98*] and others may have been treating adynamia episodica hereditaria (which is aggravated by KCl) rather than periodic paralysis [*31*] Many observers have felt that the daily administration of KCl serves a useful prophylactic function [*17 136*] especially if KCl is administered at night [*17*] Others who have been enthusiastic about the effectiveness of KCl during attacks have expressed scepticism about its prophylactic efficacy and have even observed more frequent attacks when daily treatment with potassium chloride has been provided [*16 21 25*] Such a deleterious effect might conceivably result from stimulation of increased aldosterone output if these patients react like normal subjects to a high potassium intake [*171 172*]

The use of a 10 mEq sodium intake desalting' with diuretics (chlorothiazide, mercurials acetazoleamide), and quantitative replacement of potassium losses has been very effective in preventing both spontaneous paralysis and precipitation of attacks by the most potent of exciting stimuli including glucose and insulin and 2α methyl 9α fluorohydrocortisone Conn et al [23 24 117] have made these observations in two severe and in two mild cases under strict supervision in a metabolic ward One of the severely affected patients had thyrotoxicosis after thyroidectomy he has required no sodium restriction or other treatment to avoid further seizures The two patients who were mildly affected experiencing attacks less than once a month have both remained free of attacks at home over the past 6 months and 2 years respectively on a 10 mEq sodium diet (including salt free bread and cheese no milk and with supplementary calcium gluconate and vitamins) The patient with severe and frequent attacks of paralysis has frequently admitted his inability to adhere to the low odium dietary regimen and has resorted to taking Randall s solution of potassium salts every day He continues to experience many mild bouts of weakness and an attack of paralysis lasting several hours approximately once a week It seems likely that there will be many such patients with severe forms of the disease who will fail to benefit from attempted sodium restriction because of dietary difficulties preventing strict adherence to the regimen Under such circumstances the treatment will probably fail to reduce the sodium content of the skeletal mu cle sufficiently to prevent paralysis resulting from a sudden influx of either sodium or potassium Drugs (the steroidal spirolactones) are now becoming available These inhibit the effects of circulating aldosterone and are successful in protecting patients from induced attacks of paralysis over the short periods of time that they have been used in these studies It may be that chronic administration of these or similar agents may become a practicable form of therapy if the drugs prove to be well tolerated when administered over long periods of time

Since in most patients there is a natural tendency for paralytic seizures to become less frequent and le s severe after the age of 30 or 40 years the mildly and moderately severely afflicted individuals may be treated satisfactorily with sodium restriction intermittent use of oral diuretics plus potassium replacement and perhaps aldosterone antagonists On the other hand periodic paralysis has proved to be lethal to 10 per cent of the sufferers [49] while many others have developed progressive muscular atrophy often incapacitating In families (such as that reported by Holtzapple [60] and by Myers [61]) with a high incidence of severely afflicted patients the untried and as yet unrecommended use of subtotal adrenalectomy might be considered This however remains in the realm of experimental medicine

Patients who have coexisting periodic paralysis and thyrotoxicosis are

treated with complete success by elimination of the thyrotoxicosis, by appropriate use of propylthiouracil, subtotal thyroidectomy, or radio active iodine [*53 55, 94, 98*]

## SUMMARY

1 Periodic paralysis is a disease characterized by muscular paralysis without loss of consciousness speech or sensory modalities, in persons who enjoy good health and strength between attacks Paralytic attacks commonly start between the ages of 7 and 21 years reach maximum intensity and frequency in the third decade of life and frequently tend to subside in middle or old age The frequency of attacks varies from once in a lifetime to every night for many years Males are more frequently and more severely involved than females

2 Attacks of paralysis may be induced by a variety of "stresses by factors which increase the rate of glucose utilization by rest and muscular relaxation particularly at night and by the administration of epinephrine desiccated thyroid corticotropin adrenocortical extract and mineralocorticoids

3 Prodromata are usually absent The flaccid paralysis with which the subjects characteristically awaken from sleep may be partial or complete but rarely involves facial and respiratory muscles Tendon reflexes and muscular reactivity to electrical stimulation are abolished during paralysis The frequent occurrence of cardiomegaly hypertension, bradycardia and arrhythmias and nausea vomiting constipation and reduced bowel sounds during paralysis of skeletal muscles suggests that the defect may involve the myocardium and smooth muscle as well Severe thirst and increased fluid intake often precede attacks Oliguria is usual during paralysis Recovery is frequently heralded by severe diuresis and sweating

4 Serum potassium concentration may be normal or lowered between attacks but always falls abruptly as an attack of paralysis develops This fall is not due to external losses of potassium from the body Urinary potassium output is reduced in parallel with and presumably because of the falling serum potassium concentration An increased arteriovenous difference in serum potassium concentration before and during attacks has suggested that increased uptake of potassium by the tissues constitutes a cardinal feature of the paralysis Determinations of the potassium and sodium content of biopsied skeletal muscle have confirmed a shift of potassium into the muscle without measurable change in intra muscular sodium concentration during paralysis

5 Muscular strength is increased by potassium chloride administration but only after a delay when the arteriovenous difference in serum potassium concentration indicates that potassium is being lost from the tissues Between attacks the potassium concentration in the muscles

is lowered and the sodium concentration is raised as in primary aldosteronism and other conditions of chronic potassium depletion The exchangeable body potassium tends to be low and the exchangeable body sodium tends to be high Retention of sodium and chloride usually precedes and accompanies both spontaneous and induced attacks The high sodium content of the muscles probably predisposes them to paralysis since reduction of the sodium concentration, without change in the potassium concentration by 'desalting' with a low sodium diet diuretics and potassium replacement frequently renders the muscles immune to the paralytic effects of glucose and insulin and of mineralocorticoid administration

6 Attacks of paralysis are preceded by or associated with increased urinary excretion of aldosterone and of 17 ketosteroids and less consistently of 17 hydroxycorticoids The increased glucocorticoid activity and 17 ketosteroid excretion are an integral part of the spontaneous paralytic attacks and do not occur in severe attacks induced by DCA and by ammonium glycyrrhizinate Increased glucocortocoid secretion is not essential for the induction of paralysis

7 Increase in aldosterone or aldosterone like activity is the only known factor which is common to practically all the means whereby paralysis may be induced it therefore probably plays a significant role in the pathogenesis of paralysis Sodium deprivation which abolishes most if not all of the physiologic effects of aldosterone affords protection from paralytic attacks

8 The administration of glucose and insulin increases aldosterone output in normal subjects as in patients with periodic paralysis It is probable that patients with periodic paralysis have neither adrenocortical adenomata nor any other intrinsic abnormality in their adrenal glands as the scanty autopsy evidence would confirm Presumably periodic stimuli to adrenocortical hypersecretion of aldosterone—and other steroids—occur in patients with periodic paralysis

9 The pathogenesis of paralytic seizures can be explained without postulating a primary defect in the muscle membrane but it is also possible that a defect in the normal partitioning of sodium or potassium between muscle and extracellular fluid might constitute the trigger which stimulates periodic hypersecretion of aldosterone in this condition

10 The defect in periodic paralysis might consist in intermittent excessive stimuli from the hypothalamus to the secretion of aldosterone and of the pituitary *tropic hormones This is suggested by the increased* urinary excretion of follicle stimulating hormone reported during attacks of periodic paralysis the similarity of the condition in thyrotoxic and euthyroid patients and the reported occurrence of periodic paralysis in patients with concomitant manifestations of hypothalamic disorders

11 When death has occurred during a paralytic attack it has usually

been due to respiratory causes Autopsies and muscle biopsies have been largely unrevealing except for showing the presence of characteristic vacuoles within muscle fibers Some of these are filled with granules which stain like glycogen others appear to be filled with fluid

12 *In many instances* periodic paralysis is clearly inherited as an autosomal dominant trait usually but not always with complete penetrance A sporadic form almost certainly exists also Families with periodic paralysis may have an increased incidence of migraine epilepsy and progressive muscular atrophy

## BIBLIOGRAPHY


1 Musgrave W A periodical palsy The Philosophical Transactions and Collections to the end of the year 1700 London 3 33 1727
2 Seiler Periodische Lahmung Arch med Erfahr 1, 117 1815 Cited by Talbott (ref 49)
3 Cavare *Observation d une paralysie générale du sentiment et du mouvement* affectant le type intermittent Administration du sulfate de quinine guérison Gaz hôp 26 359 1853 Also Monit hôp 1 72? 1853 Cited by Talbott (ref 49)
4 Hartwig H Ueber einen Fall von intermittirender Paralysis spinalis Zentralbl med Wiss 13 428 1875
5 Shakhnovitch On a case of intermittent paraplegia Russk Vrach 32 537 188? abstracted in London M Rec 12 130 1884
6 Westphal C Ueber einen merkwurdigen Fall von periodischer Lahmung aller vier Extremitaten mit gleichzeitigem Erlöschen der elektrischen Erregbarkeit wahrend der Lahmung Berl klin Wchnschr 22 489 509 1885
7 Goldflam S Ueber eine eigenthumliche Form von periodischer familiarer wahrscheinlich auto intoxicatorischer Paralyse Wien med Presse 31 1418 1457 1500 1536 1890
8 Goldflam S Weitere Mittheilung über die paroxysmale familiäre Lähmung Deutsche Ztschr Nervenh 7 1 1895
9 Kramer W W Zur Frage der periodischen Paralyse der Extremitaten Russ med Rundschau 6 453 517 1908
10 Shinosaki T Klinische Studien uber die periodische Extremitatenlahmung Ztschr ges Neurol u Psychiat 100 564 1926
11 Kitamura R Nihonnaukagakukai—Zasshi 1 Cited by Shinosaki [10]
12 Biemond A and Daniels A P Familial periodic paralysis and its transition into spinal muscular atrophy Brain 57 91 1934
13 Aitken R S Allott E N Castleden L I M and Walker M Observations on a case of familial periodic paralysis Clin Sc 3 47 1937
14 Singer H D and Goodbody F W A case of family periodic paralysis with a critical digest of the literature Brain 24 257 1901
15 Mitchell J K Flexner S and Edsall D L A brief report of the clinical physiological and chemical study of three cases of family periodic paralysis Brain 25 109 1902
16 Allott E N and McArdle B *Further observations on familial periodic paralysis* Clin Sc 3 229 1938
17 Gammon G D Austin J H Blithe M D and Reid C G The relation of potassium to periodic family paralysis Am J M Sc 197 326 1939
18 Ferrebee J W Gerity M K Atchley D W and Loeb R F Behavior of electrolytes in familial periodic paralysis Arch Neurol & Psychiat 44 830 1940
19 Grob D Johns R J and Liljestrand A Potassium movement in patients with familial periodic paralysis relationship to the defect in muscle function Am J Med 23 356 1957

20 Zierler K L and Andres R Movement of potassium into skeletal muscle during spontaneous attack in family periodic paralysis J Clin Invest 36 730 1957
21 Jantz H Stoffwechseluntersuchungen bei paroxysmaler Lahmung Nervenarzt 18 310 1947
22 McArdle B Le role du potassium dans la paralysie périodique familiale Semaine hop Paris 30 3724 1954
23 Conn J W Fajans S S Louis L H Streeten D H P and Johnson R D Intermittent aldosteronism in periodic paralysis dependence of attacks on retention of sodium and failure to induce attacks by restriction of dietary sodium Lancet 1 802 1957
24 Conn J W Louis L H Fajans S S Streeten D H P and Johnson R D Dependence of attacks of periodic paralysis upon retention of sodium and failure to induce either paralysis or sequestration of potassium when dietary sodium is restricted Tr A Am Physicians 70 167 1957
25 Conn J W Streeten D H P Fajans S S Louis L H and Johnson R D Adrenocortical and metabolic changes in periodic paralysis (to be published)
26 Buzzard E F Three cases of family periodic paralysis with a consideration of the pathology of the disease Lancet 2 1564 1901
27 Kulneff N Myatonia periodica Nord med Ark 35 II (Inre Med) Hft 2 1 Hft 3 20 1902
28 Favill J and Rennick C F A case of family periodic paralysis Arch Neurol & Psychiat 11 674 1924
29 Schoenthal L Family periodic paralysis with a review of the literature Am J Dis Child 48 799 1934
30 Gillespie J B Periodic paralysis Arch Pediat 54 569 1937
31 Benedek L and von Angyal L Beiträge zur Pathogenese der paroxysmalen Lähmung (Myoplegia familiaris) Ztschr ges Neurol u Psychiat 174 213 1942
32 Wolf A The effective use of thyroid in periodic paralysis New York J Med 43 1951 1943
33 Watson C W Familial periodic paralysis report of a case showing no changes in serum potassium level with a description of electroencephalographic findings Yale J Biol & Med 19 127 1946
34 G D H Family periodic paralysis Lancet 2 433 1948
35 Wyllie W G and Watkins A G Periodic familial paralysis Proc Roy Soc Med 41 861 1948
36 Lewis B A case of familial paralysis J Roy Nav M Serv 36 163 1950
37 Tyler F H Stephens F E Gunn F D and Perkoff G T Studies in disorders of muscle VII Clinical manifestations and inheritance of a type of periodic paralysis without hypopotassemia J Clin Invest 30 492 1951
38 Clinical Conference Familial periodic paralysis J Pediat 42 751 1953
39 Stevens J R Familial periodic paralysis myotonia progressive amyotrophy and pes cavus in members of a single family A M A Arch Neurol & Psychiat 72 7 6 1954
40 Sagild U and Helweg Larsen H F Det kliniske billede ved arvelige transitoriske muskellammelser periodisk adynami og periodisk paralyse Nord med 53 981 1955
41 McArdle B Familial periodic paralysis Brit M Bull 12 226 1956
42 Gamstorp I Adynamia episodica hereditaria Acta pediat Suppl 108 1 1956
43 Kaplan M Straus I Grumbach R and Aymard B Une forme particulière de paralysie périodique familiale adynamia episodica hereditaria Arch franç péd at 14 1037 1957
44 Goldflam S Ueber eine eigenthümliche Form von periodischer familiärer wahrscheinlich autointoxicatorischer Paralyse Ztschr klin Med 19 Suppl Hft 240 1891
45 Taylor E W Family periodic paralysis with a report of cases hitherto published J Nerv & Ment Dis 25 637 719 1898
46 Schmidt A K E De paroxysmale Lähmung Monograph ges Neurol u Psychiat 18 1 1919

47 Janota O and Weber K Die paroxysmale Lähmung eine Studie über ihrer Klinik und Pathogenese Abhandl Neurol Psychiat Psychol 46 1 1928
48 Dalinghaus F A Die periodische oder paroxysmale Lahmung (Myoplegia paroxysmalis) Zentralbl ges Neurol u Psychiat 100 1 1941
49 Talbott J H Periodic paralysis a clinical syndrome Medicine 20 85 1941
50 deBruin Paroxysmale verlamming Nederl tijdschr geneesk 77, 959 1933
51 Gelbard A Porazenie okresowe kónczyn z zespolem podwzgórzowoprzysadkowym Neurol polska 21 206 1938
52 Blaurock M F Studies on familial periodic paralysis Arch Neurol & Psychiat 43 416 1940
53 Dunlap H F and Kepler E J A syndrome resembling familial periodic paralysis occurring in the course of exophthalmic goiter Endocrinology 15 541 1931
54 Mora J M Periodic paralysis occurring in the course of exophthalmic goiter Endocrinology 16 407 1932
55 Weissman L Periodic paralysis associated with thyrotoxicosis a case report J Clin Endocrinol 12 1223 1952
56 Mankowsky B N Über die paroxysmale Paralyse Arch Psychiat 87 780 1929
57 MacLachlan T K Familial periodic paralysis A description of six cases occurring in three generations of one family Brain 55 47 1932
58 Khan M Y Familial periodic paralysis Indian M Gaz 70 28 1935
59 Bickerstaff E R Periodic paralysis J Neurol Neurosurg & Psychiat 16 178 1953
60 Holtzapple G E Periodic paralysis J A M A 46 1224 1905
61 Myers W A Familial periodic paralysis as traced in one family for five generations Pennsylvania M J 52 1060 1949
62 Johnson J W Jr Familial periodic paralysis report on father and son in World Wars I and II War Med 8 382 1945
63 Gaupp R Jr Erblichkeitsuntersuchungen bei paroxysmaler Lähmung Ztschr ges Neurol u Psychiat 170 108 1940
64 Fulawski A La paralysie totale des quatre extrémités et du tronc durant 48 heures Gaz hebd méd chir 27 570 1890
65 Putnam J J A case of family periodic paralysis Am J M Sc 119 160 1900
66 Crafts L M A fifth case of family periodic paralysis Am J M Sc 119 651 1900
67 Sugár M Ueber einen Fall von nicht hysterischer periodischer Lahmung Wien klin Wchnschr 23 1613 1910
68 Gardner H W A case of periodic paralysis Brain 35 243 1912–1913
69 Kirk E and Møller E Studies on a case of paroxysmal myoplegia Acta med scandinav 80 64 1933
70 Cousot G Cas de paralysie périodique Bull Acad roy méd Belg 3 s 20 791 1886
71 Zabriskie E G and Frantz A M Familial periodic paralysis Bull Neurol Inst New York 2 57 1932
72 Serko A Ein Fall von familiarer periodischer Lähmung (Oddo-Audibert) Wien klin Wchnschr 32 1138 1919
73 Infeld M Ein Fall von periodischer Lahmung Wien klin Wchnschr 18 327 1905
74 Stewart H J Smith J J and Milhorat A T Electrocardiographic and serum potassium changes in familial periodic paralysis Am J M Sc 199 789 1940
75 Streeten D H P and Conn J W Unpublished observations
76 Infeld M Einen jungen Mann mit periodischer schlaffer Lahmung aller Extremitaten Berl klin Wchnschr 50 2065 1913
77 Wexberg L Eine neue Familie mit periodischer Lahmung Jahrbücher Psychiat u Neurol 37 108 1917
78 Ziegler L H Periodic paralysis of extremities M Clin N Amer 13 1372 1930
79 Atwood C E A brief report of three cases of family periodic paralysis New York J Med 12 579 1912

80 Dalinghaus E A Beobachtung von paroxysmaler (periodischer) Lahmung bei 2 Brudern Nervenarzt 14 347 1941
81 Wilson S A K and Bruce A N *Neurology* 2d ed p 1704 Williams & Wilkins Baltimore 1955
82 Bornstein M Ueber die paroxysmale Lähmung (Versuch einer Theorie) Deutsche Ztschr Nervenh 35 407 1908
83 Kastan M Ueber einige allgemein als familiar bekannte Nervenkrankheiten (Paramyotomie ihre Kombination mit Dystrophie periodische Lähmung) Arch Psychiat 63 361 1921
84 Donath J Ein Fall von traumatischer periodischer Lahmung Wien klin Wchnschr 13 37 1900
85 Greidenberg B S Ein Fall periodischer spinaler Lähmung Russk vrach No 48 1887 abstracted in Neurol Centralbl 7 54 1888
86 Holmes J M Familial periodic paralysis Brit M J 1 80 1941
87 Tsuji K Über die Pathogenese der paroxysmalen Extremitatenlahmung Klin Wchnschr 18 370 1939
88 Bender J A Family periodic paralysis in a girl aged seventeen Arch Neurol & Psychiat 35 131 1936
89 Ziegler D K Familial periodic paralysis report on two families with observations on the pathogenesis of the syndrome Arch Int Med 84 419 1949
90 Neel A V Zwei von einander unabhangige Falle von Myoplegia paroxysmatica (periodica familiaris) Ztschr ges Neurol u Psychiat 118 269 1928
91 Kaufmann Bericht über einen sporadischen Fall von periodischer Lahmung Zentralbl ges Neurol u Psychiat 22 183 1920
92 Sugimoto E Über einen Fall von periodischer Extremitatenlahmung Folia endocrinol jap 14 65 1938 abstracted in Zentralbl ges Neurol u Psychiat 93 408 1939
93 Oliver C P Ziegler M and McQuarrie I Hereditary periodic paralysis in a family showing varied manifestations Am J Dis Child 68 308 1944
94 Linder M A Periodic paralysis associated with hyperthyroidism report of three cases Ann Int Med 43 241 1955
95 Pudenz R H McIntosh J F and McEachern D The role of potassium in familial periodic paralysis J A M A 111 2253 1938
96 Fichler W Jantz H and Jung R Elektromyographische Untersuchungen über die paroxysmale Lähmung Ztschr ges Neurol u Psychiat 170 531 1940
97 Brisset C Paralysie périodique de Westphal Sept cas dans une famille Rev neurol 88 45 1953
98 Hildebrand A G and Kepler E J Familial periodic paralysis associated with exophthalmic goiter J Nerv & Ment Dis 94 713 1941
99 Fischl J Ueber einen Fall von periodische auftretender Lahmung der unteren Extremitäten Prag med Wchnschr 10 401 1885
100 Mailhouse M Some observations on an isolated case of family periodic paralysis J Nerv & Ment Dis 37 201 1910
101 Vernon S Family periodic paralysis use of tissue extract New York J Med 36 1310 1936
102 Smith W A Periodic paralysis report of 2 fatal cases J Nerv & Ment Dis 90 210 1939
103 Gass H Cherkasky M and Savitsky N Potassium and periodic paralysis metabolic study and physiological considerations Medicine 27 105 1948
104 Adie W J A case of familial periodic paralysis Brain 50 73 1927
105 Guillain G and Barré J A Sur un cas de paralysie périodique Ann méd 6 386 1919
106 Oppenheim H Neue Mittheilungen über den von Professor Westphal beschriebenen Fall von periodischer Lähmung aller vier Extremitäten Charité-Ann 16 350 1891
107 Strauss H Zur Kasuistik der paroxysmalen Lähmung Deutsche med Wchnschr 58 1408 1932
108 Vastola E F and Bertrand C A Intracellular water and potassium in periodic paralysis Neurology 6 523 1956

109 Van Buchem F S The electrocardiogram and potassium metabolism electrocardiographic abnormalities in primary aldosteronism and familial periodic paralysis Am J Med 23 376 1957
110 Orzechowski Über die künstliche Erzeugung der sogen Periodischen Lähmungen I Kongress polnischer Neurologen Psychiater und Psychologen Warsaw 1909 abstracted in Neurol Centralbl 29 622 1910
111 Pelz Fall von periodischer Lähmung Berl klin Wchnschr 55 486 1918
112 Fuchs A Periodische Extremitätenlähmung (Oppenheim) Myoplegie (Oddo-Audibert) Myatonia periodica (Kulneff) Wien klin Wchnschr 18 21 1905
113 Poulson J E Et tilfaelde af paroxysmatisk myoplegi Ugesk laeger 100 998 1938
114 Cerny A and Katzenstein Sutro L Die paroxysmale Lähmung Schweiz Arch Neurol u Psychiat 70 259 1952
115 Ziegler M R and McQuarrie I Hereditary periodic paralysis effects of various induced changes in physiological state on paralytic attacks Metabolism 1 116 1952
116 Morrison S and Levy M The thyroid factor in family periodic paralysis report of a cas Arch Neurol & Psychiat 28 386 1932
117 Conn J W Streeten D H P Fajans S S Louis L H and Johnson R D Unpublished observations
118 Schlesinger H Ueber die periodisch auftretende (paroxysmale) Lähmung Wien klin Wehnschr 18 3 3 1905
119 Danowski T S Elkinton J R Burrows B A and Winkler A W Exchanges of sodium and potassium in familial periodic paralysis J Clin Invest 27 65 1948
119a Hondkarjan O A Paroxysmale muskellähmung Nevropat i Psihiat 7 116 1938 abstracted in Zentralbl ges Neurol u Psychiat 93 410 1939
120 Eales L Linder G C and Sakinofsky I Sodium and potassium balance in relation to periodic paralysis and in a case of pyelonephritis with malignant hypertension South African M J 32 251 1958
121 Edsall D L and Means J H Observations on a case of family periodic paralysis Am J M Sc 150 169 1915
122 Louis L H Unpublished observations
123 Milhorat A T and Toscani V Studies in diseases of muscle metabolism of calcium phosphorus and magnesium in progressive muscular dystrophy myotonia atrophica and familial periodic paralysis Arch Neurol & Psychiat 41 1130 1939
124 Yoshimura K Zur Kenntnis der periodischen Extremitätenlähmung deren Ursache und Therapie München med Wchnschr 76 1971 1929
125 Grob D Liljestrand A and Johns R J Potassium movement in normal subjects effect on muscle function Am J Med 23 340 1957
126 Hajdu S Mechanism of staircase and contracture in ventricular muscle Am J Physiol 174 371 1953
127 Darrow D C Schwartz R Iannucci J F and Coville F The relation of serum bicarbonate concentration to muscle composition J Clin Invest 27 198 1948
128 Szent Györgyi A *Chemical Physiology of Contraction in Body and Heart Muscle* Academic Press Inc New York 1953
129 Sagild U Total exchangeable potassium in normal subjects with special reference to changes with age Scandinav J Clin & Lab Invest 8 44 1956
130 McArdle B and Merton P A The behaviour of radio potassium in man J Physiol 116 51 1952
131 Robinson C V Arons W L and Solomon A K An improved method for simultaneous determination of exchangeable body sodium and potassium J Clin Invest 34 134 1955
132 deGraeff J and Brocker J S H Intermittent aldosteronism in periodic paralysis Lancet 1 1130 1958
133 Bartter F C Liddle G W Duncan L E Jr Barber J K and Delea C

The regulation of aldosterone secretion in man the role of fluid volume J Clin Invest 35 1306 1956
134 Beck J C Dyrenfurth I Giroud C P and Venning E H Observations on the regulatory mechanisms of aldosterone secretion in man A M A Arch Int Med 96 463 1955
135 Muller A F Riondel A M and Mach R S Control of aldosterone excretion by changes in volume of body fluid Lancet 1 831 1956
136 Lales L Personal communication
137 McQuarrie I and Ziegler M R Hereditary periodic paralysis effects of fasting and of various types of diet on occurrence of paralytic attacks Metabolism 1 1 9 195
138 Silber R H and Porter C C The determination of 17 21-dihydroxy 0-ketosteroids in urine and plasma J Biol Chem 210 9 3 1954
139 *Medical Research Council Committee on Clinical Endocrinology* Proposed standard method of 17 ketosteroid determination Lancet 2 585 1951
140 Streeten D H P Conn J W Louis L H Fajans S S Seltzer H S Johnson R D Gittler R D and Dube A H Secondary aldosteronism the metabolic and adrenocortical responses of normal men to high environmental temperatures Metabolism (in press)
140a Neher R and Wettstein A Physicochemical estimation of aldosterone in urine J Clin Invest 35 800 1956
141 Cope C L and Garcia Llaurado J The occurrence of electrocortin in human urine Brit M J 1 1290 1954
142 Liddle G W Richard J L and Tomkins G M Studies of structure function relationships of steroids the 2 methyl-corticosteroids Metabolism 5 384 1956
143 Hirschowitz B I and Streeten D H P Significance of increased urinary pepsinogen (uropepsin) excretion during ACTH administration and in duodenal ulcer patients J Lab & Clin Med 50 209 1957
144 Hirschowitz B I Streeten D H P London J A and Pollard H M Effects of eight hour intravenous infusions of ACTH and the adrenocortical steroids in normal man I Basal gastric secretion and plasma and urinary pepsinogen J Clin Invest 36 1171 1957
144a Crane M G Vogel I J and Richland K J Observations on a presumptive case of primary aldosteronism J Lab Clin Med 48 1 1956
145 Venning L H Dyrenfurth I and Beck J C Effect of anxiety upon aldosterone excretion in man J Clin Endocrinol 17 1005 1957
146 Llaurado J G Increased excretion of aldosterone immediately aft r operation Lancet 1 1295 1955
147 Liddle G W Duncan L E Jr and Bartter F C Dual mechanism regulating adrenocortical function in man Am J Med 21 380 1956
148 Falbriard A Muller A F Neher R and Mach R S Étude des variations de l aldostéronurie sous l effet de surcharges en potassium et de d'perditions rénales et extrarénales de sel et d eau Schweiz med Wchnschr 85 1218 1955
149 Bartter F C and Biglieri E G Primary aldosteronism clinical staff conference at the National Institutes of Health Ann Int Med 48 647 1958
150 Kagawa C M Cella J A and van Arman C G Action of new steroids in blocking effects of aldosterone and desoxycorticosterone on salt Science 126 1015 1957
151 Liddle G W Sodium diuresis induced by steroidal antagonists of aldosterone Science 126 1016 1957
152 Conn J W Louis L H Fajans S S Streeten D H P Johnson R D Moorhouse J R Crane M G Berker A F and Ramirez E Metabolic effects in normal men and in primary aldosteronism of a synthetic aldosterone antagonist J Lab A Clin Med 52 805 1958
153 Farrell G Regulation of aldosterone secretion Physiol Rev 38 09 1958
154 Conn J W Presidential address painting background primary aldosteronism a new clinical syndrome J Lab & Clin Med 45 3 1955
155 Conn J W, McGehee J M Johnson R D Louis L H and Streeten

109 Van Buchem I S The electrocardiogram and potassium metabolism electrocardiographic abnormalities in primary aldosteronism and familial periodic paralysis Am J Med 23 376 1957
110 Orzechowski Über die künstliche Erzeugung der sogen Periodischen Lähmungen I Kongress polnischer Neurologen Psychiater und Psychologen Warsaw 1909 abstracted in Neurol Centralbl 29 622 1910
111 Pelz Fall von periodischer Lähmung Berl klin Wchnschr 55 486 1918
112 Fuchs A Periodische Extremitätenlähmung (Oppenheim) Myoplegie (Oddo-Audibert) Myatonia periodica (Kulneff) Wien klin Wchnschr 18 21 1905
113 Poulson J L Et tilfaelde af paroxysmatisk myoplegi Ugesk laeger 100 998 1938
114 Cerny A and Katzenstein Sutro E Die paroxysmale Lähmung Schweiz Arch Neurol u Psychiat 70 259 1952
115 Ziegler M R and McQuarrie I Hereditary periodic paralysis effects of various induced changes in physiological state on paralytic attacks Metabolism 1 116 1952
116 Morrison S and Levy M The thyroid factor in family periodic paralysis report of a case Arch Neurol & Psychiat 28 386 1932
117 Conn J W Streeten D H P Fajans S S Louis L H and Johnson R D Unpublished observations
118 Schlesinger H Ueber die periodisch auftretende (paroxysmale) Lähmung Wien klin Wchnschr 18 373 1905
119 Danowski T S Elkinton J R Burrows B A and Winkler A W Exchanges of sodium and potassium in familial periodic paralysis J Clin Invest 27 65 1948
119a Hondkarjan O A Paroxysmale muskellähmung Nevropat i Psihiat 7 116 1938 abstracted in Zentralbl ges Neurol u Psychiat 93 410 1939
120 Eales L Linder G C and Sakinofsky I Sodium and potassium balance in relation to periodic paralysis and in a case of pyelonephritis with malignant hypertension South African M J 32 251 1958
121 Edsall D L and Means J H Observations on a case of family periodic paralysis Am J M Sc 150 169 1915
122 Louis L H Unpublished observations
123 Milhorat A T and Toscani V Studies in diseases of muscle metabolism of calcium phosphorus and magnesium in progressive muscular dystrophy myotonia atrophica and familial periodic paralysis Arch Neurol & Psychiat 41 1130 1939
124 Yoshimura K Zur Kenntnis der periodischen Extremitätenlähmung deren Ursache und Therapie München med Wchnschr 76 1971 1979
125 Grob D Liljestrand A and Johns R J Potassium movement in normal subjects effect on muscle function Am J Med 23 310 1957
126 Hajdu S Mechanism of staircase and contracture in ventricular muscle Am J Physiol 174 371 1953
127 Darrow D C Schwartz R Iannucci J F and Coville F The relation of serum bicarbonate concentration to muscle composition J Clin Invest 27 198 1948
128 Szent Györgyi A *Chemical Physiology of Contraction in Body and Heart Muscle* Academic Press Inc New York 1953
129 Sagild U Total exchangeable potassium in normal subjects with special reference to changes with age Scandinav J Clin & Lab Invest 8 44 1956
130 McArdle B and Merton P A The behaviour of radio-potassium in man J Physiol 116 51 1952
131 Robinson C V Arons W L and Solomon A K An improved method for simultaneous determination of exchangeable body sodium and potassium J Clin Invest 34 134 1955
132 deGraeff J and Brocker J S H Intermittent aldosteronism in periodic paralysis Lancet 1 1130 1958
133 Bartter F C Liddle G W Duncan L E Jr Barber J K and Delea C

Chapter 28

# Adynamia Episodica Hereditaria

*Jerome W Conn and David H P Streeten*

The literature of the past 60 years contains a number of hints recognizable in retrospect of differences between the usual cases of classical periodic paralysis and the group recently described as adynamia episodica hereditaria [1] Tyler et al [2] were the first to appreciate that because the serum potassium level always fell during attacks in patients with the usual periodic paralysis the consistent absence of hypokalemia in a family which they studied indicated the existence of at least two types of the disorder These authors considered however that the clinical features in their patients were indistinguishable from those of periodic paralysis This opinion was strongly supported by numerous published case reports of patients clinically identical with theirs described as having periodic paralysis Sagild et al [3] described a similar family and McArdle [4] referring to a family that he had studied suggested the need for a separate name for this group of patients Gamstorp and Mjones [1] were the first to draw attention to a number of clinical features such as the absence of changes in serum potassium level and in the electrocardiogram during attacks which prompted them to delineate from the main group of patients with periodic paralysis a group whose disorder they proposed to name adynamia episodica hereditaria

In these patients the attacks of weakness usually started at an earlier age than in periodic paralysis occurred more often by day than by night were less widespread less severe and shorter The attacks tended to be precipitated by hunger and by KCl administration and to be relieved by the ingestion of food In a comprehensive review of the previous literature Gamstorp [5] found no less than 13 reports [2 3 6–16] of individuals or families who e clinical manifestations and normal potassium concentration during attacks strongly sugge ted the diagno is of adynamia episodica hereditaria rather than that of classical periodic paralysis

D H P Observations on a case of periodic paralysis with thyrotoxicosis (in preparation)
156 Crane M G Personal communication
157 Grossman C Periodic paralysis associated with obesity of hypothalamic origin observations in case with changes in electrocardiogram and serum potassium level Arch Neurol & Psychiat 62 105 1949
158 Woodbury D M and Koch A Effects of aldosterone and desoxycorticosterone on tissue electrolytes Proc Soc Exper Biol & Med 94 720 1957
159 Neustaedter M A case of periodic family paralysis New York J Med 22 65 1922
160 Roger H and Schachter M Étude clinique de deux cas de paralysie périodique familiale maladie de Westphal Monatschr Psychiat u Neurol 112 169 1946
161 Saunders M G Electroencephalograph findings in a case of familial periodic paralysis with hypopotassemia Electroencephalog & Clin Neurophysiol 6 499 1954
162 Gates R R *Human Genetics* vol 2 p 973 Macmillan New York 1946
163 Gaupp R Jr and Kalden O Erblichkeitsuntersuchungen bei paroxysmaler Lähmung II Mitteilung Ztschr ges Neurol u Psychiat 174 194 1942
164 Samuelsohn Fall von intermittirender Paraplegie Berl klin Wchnschr 14 607 1877
165 Bornstein M Asthenia paroxysmalis Neurol Centralbl 24 696 750 1905
166 Johnsson V Über paroxysmale Lähmung Hygiea 93 219 1931
167 Skouge E Über paroxysmale periodische Lähmung Norsk Mag Laegevidensk 93 1007 1932 abstracted in Zentralbl ges Neurol u Psychiat 66 88, 1933
168 Veger A M and Yeroukhimovitch B M Zur Klinik der periodischen Lähmungen Sovet Psichoneur 12 89 1936 abstracted in Zentralbl ges Neurol u Psychiat 86 593 1937
169 Maclaire A S A case of periodic paralysis associated with thyroid enlargement J Nerv & Ment Dis 61 44 1925
170 Dunlap H F and Kepler E J Occurrence of periodic paralysis in the course of exophthalmic goiter Proc Staff Meet Mayo Clin 6 272 1931
171 Laragh J H and Stoerk H C On the mechanism of secretion of the sodium retaining hormone (aldosterone) within the body J Clin Invest 34 913 1955
172 Myers W A Personal communication
172a Liddle G W Bartter F C Duncan L E Jr Barber J K and Delea C Mechanisms regulating aldosterone production in man J Clin Invest 34 949 1955
173 Shohl A T *Mineral Metabolism* p 29 Reinhold New York 1939
174 Farago P J Rochlin I Schilling R C Vawter G F and Armstrong S H Jr in *Proceedings of the Second Clinical A C T H Conference* vol 1 p 213 McGraw Hill—Blakiston New York 1951
175 Baldwin D Robinson P K Zierler K L and Lilienthal J L Jr Interrelations of magnesium potassium phosphorus and creatine in skeletal muscle of man J Clin Invest 31 850 1952
176 van Buchem F S P Doorenbos H and Elings H S Primary aldosteronism due to adrenocortical hyperplasia Lancet 2 335 1956

manent muscular weakness occurred in 2 of the 138 patients described by Gamstorp [5]

*Inciting Factors*

In all these patients attacks are most likely to occur during rest after severe muscular exercise [2 5–8 11–13 20] Attacks may be brought on at will in some individuals by complete relaxation at any time of the day Cold and dampness tend to bring on attacks [5 19] and it has been reported that epinephrine [18] emotional stress [2] 20 to 30 units of insulin [11] and glucose (200 gm) [18] will precipitate attacks Tyler et al [2] have pointed out that a heavy meal at night will not cause any weakness within 2 hr but will frequently precipitate an attack the next morning All these factors which precipitate attacks in adynamia episodica hereditaria may have a similar effect in periodic paralysis In contrast with periodic paralysis however it has been reported that hunger frequently excites attacks of weakness in adynamia episodica hereditaria [5 19] while the ingestion of glucose or of a meal during the day appears to ward off an impending attack [5] In contrast with periodic paralysis the administration of KCl in doses of 2 to 3 gm in children and 4 gm by mouth in adults almost always precipitates attacks [5] One wonders whether or not KCl would precipitate attacks if such patients were maintained on diets very low in sodium

## LABORATORY FINDINGS

Serum potassium has always been normal [2 10 12 18] or elevated [5 11] during attacks of weakness or paralysis whether these occurred spontaneously or were induced by KCl administration

The urinary potassium far from showing the sharp fall seen in attacks of periodic paralysis tended to rise during the seizures of adynamia episodica hereditaria [4 5 21 22]

The electrocardiogram showed slightly increased amplitude and a peaked appearance of T waves [3 5 20] reflecting the hyperkalemia during the attacks

Exchangeable potassium is said to have been normal in three patients [22] though the actual figures have not yet been published

The serum phosphorus has fallen in attacks [5] as in periodic paralysis No abnormal findings have been encountered in serum albumin globulin or cholesterol [18] sodium chloride $CO_2$ Ca and Mg [5] Hematocrit fasting blood sugar [ ] BMR [18] electroencephalogram and cerebrospinal fluid [5] have also been normal

Blood eosinophil count has been observed to fall by more than 50 per cent in 19 out of 20 instances of spontaneous attacks and in 3 out of 11 attacks precipitated by KCl [5]

Four more reports have been found [4 17–19] of a condition which fulfills the criteria laid down by Gamstorp [5] A recent report [20] of a family described as having adynamia episodica hereditaria has been published It is not known whether the underlying pathogenic mechanism differs significantly in these two groups of patients but in view of the differences in clinical features, adynamia episodica hereditaria will be considered separately here

## CLINICAL FEATURES

### *Age of Onset*

This is usually earlier in adynamia episodica hereditaria than in periodic paralysis, the condition is often recognized during early infancy Thus among 108 patients in two families described by Gamstorp [5] the attacks started in the first 5 years of life in 50 per cent and before the age of 10 years in more than 90 per cent This onset in the first decade of life contrasts with the onset in periodic paralysis which begins usually in the second or third decades as shown in Fig 27 1

### *Description of Attacks*

Attacks occurred more frequently by day than by night [5 6 9 10 20] and 27 of the 138 patients described by Gamstorp [5] never experienced nocturnal seizures Attacks occurred at night too [5 6 18] however, and tended to be more severe than the daytime seizures [20] The duration of weakness or paralysis was very variable lasting occasionally for 1 or 2 days but more frequently terminating within 10 to 45 min [5 8, 9 12] One individual (G D H ) [19] described by Buzzard [6] in 1901 as having periodic paralysis has drawn attention to two main types of attacks in himself (1) "ordinary attacks" of slight weakness involving a few or many muscle groups lasting up to an hour occurring as often as four times a day and resulting from such unpleasant experiences as hunger, cold diarrhea or severe exercise and (2) "continuing attacks" with beaten exhausted feelings in the legs lasting for several days Many authors have described the ability of these patients to "walk off" attacks [2 6 8 13 21] as can patients with periodic paralysis Severe sweating may occur during the seizures [6 8 20 21] A positive Chvostek sign was present in 25 per cent of Gamstorp's patients between attacks [5] This sign was elicited in both the patients described by Kaplan et al [20] but in one of these patients precipitation of an attack by potassium chloride administration was required to bring out the sign Tyler et al [2] have remarked upon the muscular build of a number of these patients This often gives the appearance of the pseudohypertrophy seen in childhood progressive muscular dystrophy

No deaths have been reported during paralytic seizures but per

manent mu cular weakness occurred in 2 of the 138 patients described by Gamstorp [5]

*Inciting Factors*

In all these patients attacks are mo t likely to occur during rest after severe muscular exercise [2 5–8 11–13 20] Attacks may be brought on at will in some individuals by complete relaxation at any time of the day Cold and dampness tend to bring on attacks [5 19] and it has been re ported that epinephrine [18] emotional stress [2] 20 to 30 units of insulin [11] and glucose (200 gm) [18] will precipitate attacks Tyler et al [2] have pointed out that a heavy meal at night will not cause any weakness within 2 hr but will frequently precipitate an attack the next morning All these factors which precipitate attacks in adynamia episodica hered itaria may have a similar effect in periodic paralysis In contrast with periodic paralysis however it has been reported that hunger frequently excites attacks of weakness in adynamia episodica hereditaria [5 19] while the ingestion of glucose or of a meal during the day appears to ward off an impending attack [5] In contrast with periodic paralysis the administration of KCl in doses of 2 to 3 gm in children and 4 gm by mouth in adults almost always precipitates attacks [5] One wonders whether or not KCl would precipitate attacks if such patients were maintained on diets very low in sodium

## LABORATORY FINDINGS

Serum potassium has always been normal [2 10 12 18] or elevated [5 11] during attacks of weakness or paralysis whether the e occurred spontaneously or were induced by KCl administration

The urinary potassium far from showing the sharp fall seen in attacks of periodic paralysis tended to rise during the seizures of adynamia episodica hereditaria [4 5 21 22]

The electrocardiogram showed slightly increased amplitude and a peaked appearance of T waves [3 5 20] reflecting the hyperkalemia during the attacks

Exchangeable potassium is aid to have been normal in three patients [22] though the actual figures have not yet been published

The serum pho phorus has fallen in attacks [5] as in periodic paralysis No abnormal findings have been encountered in serum albumin globulin or cholesterol [18] sodium chloride $CO_2$ Ca and Mg [5] Hematocrit fasting blood sugar [5] BMR [18] electroencephalogram and cere bro pinal fluid [5] have al o been normal

Blood eosinophil count has been ob erved to fall by more than 50 per cent in 19 out of 20 instances of spontaneous attacks and in 3 out of 11 attacks precipitated by KCl [5]

Urinary creatinine output has increased significantly on the day of the attack [3 17]

The electromyogram has revealed muscle action potentials which are always significantly shortened during paresis a change opposite to that seen in periodic paralysis (see Electromyographic and Chemical Changes in Muscle Chap 27)

All the patients studied have had an increased sensitivity to intra arterial injections of acetylcholine Thus 50 to 100 $\mu g$ acetylcholine produced a motor response in 5 patients, while 100 to 300 $\mu g$ was required in normal subjects This suggests a degree of hypopolarization of the muscle end plate both during and between attacks in adynamia episodica hereditaria

## PATHOLOGY

Tyler et al [2] have drawn attention to the presence of irregular clusters of nuclei along the more slender of the muscle fibrils and to the occurrence of vacuoles in the muscle Apparently these vacuoles are identical with those observed frequently in periodic paralysis (see Mortality and Pathologic Findings, Chap 27) Some of the vacuoles [2] contained unstained debris or appeared empty *others contained granules which* stained like glycogen with Best's stain However determination of the muscle content of glycogen has failed to show any abnormality

## PATHOGENESIS

There is little doubt that adynamia episodica hereditaria is a *clinical* entity which can be differentiated with reasonable ease from periodic paralysis Its strikingly earlier age of onset milder course and lack of incapacitating or fatal sequellae provide good practical grounds for differentiating it from periodic paralysis

On the other hand some of the other clinical differences may have been overemphasized While meals are said to improve symptoms during the day one wonders how conclusively this effect has been established in individuals whose weakness is mild and usually lasts for only 10 to 45 min without *any* treatment Furthermore it is heavy meals at *night* which are particularly likely to precipitate attacks in periodic paralysis and Tyler et al [2] have found precisely the same effect of heavy meals taken at bedtime in patients with adynamia episodica hereditaria viz attacks occurring early the next morning

The absence of a fall in serum potassium would be most unusual in severe attacks of quadriplegia occurring in patients with periodic paralysis but is certainly quite common in periodic paralysis during the mild spells of weakness which occur frequently between major bouts of paralysis This has been reported by Pudenz et al [23] Bickerstaff [24]

and others and is evident from Fig 27 5 Moreover there is considerable doubt that KCl has any therapeutic effect in such spells of transient weakness in patients with periodic paralysis The beneficial effects in periodic paralysis of potassium even in severe attacks are seldom seen within 15 min of administration and may be delayed for 2 hr or more [25] Furthermore some patients with a clinical picture typical of adynamia episodica hereditaria are stated to have experienced relief from potassium administration despite the absence of a fall in serum potassium during the weakness [18]

Evidence suggesting a similar pathogenesis in the two conditions includes (1) the tendency in both conditions for attacks to be precipitated by a heavy meal at night [2] by glucose [18] or insulin administration [11] and by emotional stress [2] all of which can be expected to stimulate an increased adrenocortical output of aldosterone (see Nature of the Relationship between Transient Hyperaldosteronism and Paralysis Chap 27) (2) the fall in blood eosinophil count [5] which probably signifies increased adrenocortical secretion of hydrocortisone during seizures of adynamia episodica hereditaria as has been shown to occur in attacks of periodic paralysis (see Laboratory Findings Chap 27) and (3) presence of the identical pathologic lesion comprising vacuoles within the muscle fibers in both conditions [2 5]

Conclusive evidence on exactly how the pathogenic mechanisms of adynamia episodica hereditaria and periodic paralysis differ and in what respects they may be identical can be expected to result from future studies of muscle electrolyte composition adrenocortical activity and the ability to precipitate paralysis with potent salt retaining corticoids in patients with adynamia episodica hereditaria

## GENETICS

Dominant autosomal inheritance with complete or almost complete penetrance has been reported by most writers [2 3 7 8 12 13 17] and is shown in Fig 28 1 Penetrance was incomplete in one family [18] The incidence of the disorder among males and females in hereditary cases has been equal [21] though Tyler et al [2] found that the symptoms were more severe in males than in females A history of migraine or severe headaches has been encountered among relatives unaffected by adynamia episodica hereditaria [8 9] Apparently genuine instances of sporadic occurrence of this condition have been reported [9 10]

## TREATMENT

Administration of potassium salts either has proved of no avail [2 11 15] or has actually aggravated the symptoms [5 12] This finding is not sur

prising in view of the ease with which attacks have been precipitated by KCl Premedication with glucose has prevented the precipitation of attacks by KCl [5]

Wolf [11] has reported very good results from the use of desiccated thyroid which given to seven patients completely protected them from attacks for 1½ years

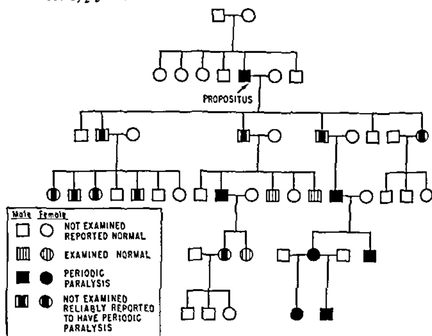



Fig 28-1 Genealogy of adynamia episodica hereditaria as exemplified by Gamstorp's family from Matterod [5]

Intravenous calcium gluconate (5 to 20 ml 10 per cent solution) appears to have shortened attacks [5] cod liver oil (500 ml per week) is claimed to have prevented all attacks from 14 days after commencement of its administration [19]

Gamstorp et al [6] have recommended a regimen of moderate physical exertion enough sleep at night and regular meals at short intervals during the day

It is to be expected that newer information regarding the pathogenesis of adynamia episodica hereditaria and its probable relationship to periodic paralysis will be forthcoming and that rational therapeutic procedures will result

## SUMMARY

1 Adynamia episodica hereditaria is delineated from classical periodic paralysis by the absence of the fall in serum potassium concentra

tion during attacks of weakness or paralysis A number of families with this condition have now been described or recognized in retrospect from reports in the literature

2 The attacks begin earlier in life than in patients with periodic paralysis They occur frequently in infancy and almost invariably within the first decade of life The attacks usually occur during the daytime and are milder and of shorter duration than in periodic paralysis They are commonly limited to slight weakness of a few or several muscle groups for 10 to 15 min

3 Inciting events include hunger and potassium administration and also *many factors* which induce attacks of periodic paralysis such as muscular relaxation previous severe exercise cold emotional stress and administration of epinephrine It is said that the ingestion of glucose or of a meal will ward off attacks but glucose insulin and a heavy meal may also precipitate paralysis after a latent period of several hours as in periodic paralysis No deaths or incapacitating sequellae have yet been reported The paretic episodes are seldom severe enough to interfere with the normal daily occupation

4 The serum potassium concentration is normal or elevated during attacks of weakness The electrocardiogram may show slight peaking of T waves Muscle action potentials are shortened Urinary potassium tends to rise during seizures Increased sensitivity of muscles to intraarterial acetylcholine suggests hypopolarization of the muscle end plate As in periodic paralysis there is a fall in serum phosphorus level and in the blood eosinophil count during seizures

5 Histologically the muscles show the same vacuolar lesion that is present in periodic paralysis These and other features common to periodic paralysis and adynamia episodica hereditaria suggest that the underlying pathogenic mechanisms may be similar but it is useful for prognostic reasons to differentiate between the two diseases

6 The disease is inherited as an autosomal dominant trait with complete or almost complete penetrance There is sometimes a history of migraine in affected families

7 *Treatment has been restricted to the recommendation of a temperate* existence and administration of calcium gluconate cod liver oil and desiccated thyroid

## BIBLIOGRAPHY

1 Gamstorp I and Mjönes H cited by Gamstorp I Adynamia episodica hereditaria Acta paediat Upps 45 Suppl 108 1 1956
2 Tyler F H Stephens F E Gunn F D and Perkoff G T Studies in disorders of muscle VII Clinical manifestations and inheritance of a type of periodic paralysis without hypopotassemia J Clin Invest 30 492 1951
3 Sagild U and Helweg Larsen H F Det kliniske billede ved arvelige transitoriske muskellammelser periodisk adynami og periodisk paralyse Nord med 53 981 1955

4 McArdle B Familial periodic paralysis Brit M Bull 12 226 1956
5 Gamstorp I Adynamia episodica hereditaria Acta paediat Upps 45 Suppl 108 1 1956
6 Buzzard L F Three cases of family periodic paralysis with a consideration of the pathology of the disease Lancet 2 1564 1901
7 Kulneff N Myatonia periodica Nord Med Ark 1907 3 F II, (Inre Med) Hft 2 Nr 10 1 and Hft 3 Nr 11 21
8 Schoenthal L Family periodic paralysis with a review of the literature Am J Dis Child 48 799 1934
9 Gillespie J B Periodic paralysis Arch Pediat 54 569 1937
10 Benedek L and von Angyal L Beiträge zur Pathogenese der paroxysmalen Lähmung (Myoplegia familiaris) Ztschr ges Neurol u Psychiat 174 213 1942
11 Wolf A The effective use of thyroid in periodic paralysis New York J Med 43 1951 1943
12 Wyllie W G and Watkins A G Periodic familial paralysis Proc Roy Soc Med 41 861 1948
13 Lewis B A case of familial paralysis J Roy Nav M Serv 42 751 1953
14 Clinical Conference Familial periodic paralysis J Pediat 42 751 1953
15 Stevens J R Familial periodic paralysis myotonia progressive amyotrophy and pes cavus in members of a single family A M A Arch Neurol & Psychiat 72 7 6 1954
16 Neel A V Zwei von einander unabhängige Fälle von Myoplegia paroxysmatica (periodica familiaris) Ztschr ges Neurol u Psychiat 118 269 1929
17 Favill J and Rennick C F A case of family periodic paralysis Arch Neurol & Psychiat 11 674 1924
18 Watson C W Familial periodic paralysis report of a case showing no changes in serum potassium level with a description of electroencephalographic findings Yale J Biol & Med 19 127 1946
19 G D H Family periodic paralysis Lancet 2 433 11 1048
20 Kaplan M Straus L Crumbach R and Aymard B Une forme particulière de paralysie périodique familiale adynamia episodica hereditaria Arch franç pédiat 14 1057 1957
21 Gamstorp I Adynamia episodica hereditaria Acta genet et statist med 7 325 1957
22 Gamstorp I Hauge M Helweg Larsen H F Mjönes H and Sagild U Adynamia episodica hereditaria a disease clinically resembling familial periodic paralysis but characterized by increasing serum potassium during paralytic attacks Am J Med 23 385 1957
23 Pudenz R H McIntosh J F and McEachern D The role of potassium in familial periodic paralysis J A M A 111 2253 1938
24 Bickerstaff E R Periodic paralysis J Neurol Neurosurg Psychiat 16 178 1953
25 Grob D Johns R J and Liljestrand A Potassium movement in patients with familial periodic paralysis relationship to the defect in muscle function Am J Med 23 356 1957

# Chapter 29

# Pseudohypoparathyroidism

---

*Frederic C. Bartter*

Pseudohypoparathyroidism (PHP) is a term introduced by Albright, Burnett, Smith, and Parson [1] to describe a syndrome in which the serum shows the abnormalities of hypoparathyroidism but which differs from "idiopathic" or surgical hypoparathyroidism in that parathyroid extract has little or no effect in correcting the abnormalities. Whereas a typical case may present numerous distinctive features not found in idiopathic hypoparathyroidism, there is no general agreement as to the precise physical or biochemical criteria required to establish a diagnosis. Although this uncertainty has led some authors to include under pseudohypoparathyroidism cases with but a single feature, and has even led a few to question the existence of the entity, an extensive and convincing body of literature now leaves little doubt that it exists as a unique syndrome and that it is a familial disease.

## CLINICAL FEATURES

Patients with PHP are almost invariably described as having short stature and round faces [2, 3] (Fig. 29.1). They show, in addition, shortening of one or more metatarsal or metacarpal bones and shortness of the corresponding digits [1–4]. Clinical or radiologic examination may reveal areas of calcification or ossification in the skin or soft tissues. There may be exostoses typical of those seen in the syndrome of hereditary deforming chondrodysplasia [5–7]. A number of cases have been reported in siblings [2, 8–12]. If one includes in the significant family history the metatarsal and metacarpal changes resembling those of achondroplasia and the presence of exostoses, the evidence for positive family history is impressive.

These findings are not characteristic of idiopathic hypoparathyroidism. On the other hand, with the single exception of associated moniliasis [13], all the findings characteristic of idiopathic hypoparathyroidism have been reported in pseudohypoparathyroidism. These include both

tetanic and epileptic convulsions fragility of the nails thickness of the skull hypoplasia of the enamel and of the roots of the teeth cataracts and prolonged Q T interval in the electrocardiogram In both syndrome the serum alkaline phosphatase level is generally normal but may be elevated [2 14 16] the bones are generally thicker than average but may indeed, be radiolucent [1, 3 11, 16, 17] and there may be scattered areas of calcification in and about the region of the basal ganglia Abnormally high serum phosphorus concentration abnormally low serum calcium concentration and a very low urinary calcium excretion are characteristics of both



Fig 29-1 Face of patient G H with pseudohypoparathyroidism (*By permission of H Elrick et al* [9])

Patients with PHP are frequently mentally retarded [1-3] and the face is frequently expressionless masklike, or suggestive of parkinsonism [3 4] In a number of reports the bones are described as showing *increased trabeculation* and coarse structure [11, 16 17] The first two features are difficult to evaluate quantitatively and bone biopsy which would allow better definition of the third has been performed in only one atypical case [14]

As originally described [1] the syndrome differed in two cardinal features from idiopathic hypoparathyroidism normal or indeed increased amounts of parathyroid tissue were demonstrated on exploration and there was resistance to administered parathyroid extract The resistance can be demonstrated by two different types of test the results of which may differ significantly

In the first reported case [1 Patient I S] parathyroid tissue appeared to be normal in amount after 6 months of treatment with dihydrotachysterol In two other cases [1 G H and S M B] biopsy of the parathyroids showed hyperplasia Figure 29 2 is a photomicrograph of the tissue removed from G H and S M B The e findings are in striking contrast to those reported for idiopathic hypoparathyroidism in which parathyroid tissue has not been found despite careful search at autopsy on a number of occasions [10 18] MacGregor and Whitehead note that in one reported case there was strong presumptive evidence for idiopathic hypoparathyroidism but normal parathyroid tissue was found at autopsy [4] In this patient a single normal serum calcium value was reported The metatarsal and metacarpal bones were not described

The literature contains a further report of a parathyroid biopsy in PHP [4] No parathyroid tissue was found and the results were termed "inconclusive" by the authors Unfortunately biopsy has not been performed in any of the remaining cases thus far reported Accordingly the diagnosis has been established on clinical grounds and with the use of

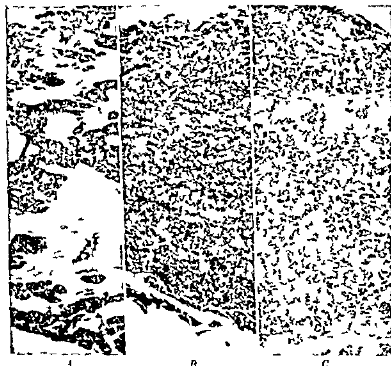



Fig 29 2 Microscopic picture of parathyroid gland A A normal subject B patient G H [ ] with pseudohypoparathyroidism C patient S M B [*] with pseudohypoparathyroidism Note the hyperplasia in (B) and (C) (X105)

parathyroid extract a procedure which as discussed below may give equivocal results

The association of chemical hypoparathyroidism with hyperplasia of the parathyroids and resistance to parathyroid extract led Albright and associates to postulate an "end-organ" defect in PHP They drew an analogy to Sebright Bantam roosters which develop the plumage of hens under androgenic stimulation

## RESPONSE TO PARATHYROID EXTRACT

Response to parathyroid extract may be tested by observing the immediate changes in urine and serum phosphorus when the extract is

given intravenously, or by observing changes in serum calcium and phosphorus and in calcium and phosphorus balance when the extract is given intramuscularly over a period of days The first type of test is generally done exactly as described by Ellsworth and Howard [20] thus it is possible to compare the quantitative results of one laboratory with those of another The second type of test has been carried out under widely varying conditions of calcium and phosphorus intake and with differing doses of parathyroid extract so that quantitative evaluation of results from different laboratories is very difficult

In the Ellsworth Howard test the patient is fasted and urine is collected for measurement of phosphorus content for 3 hr Two hundred units of parathyroid extract are then given intravenously and urine and serum phosphorus are measured for a further period of 3 hr In patients with idiopathic or surgical hypoparathyroidism urine phosphorus may rise to reach five to ten times control values and serum phosphorus may fall by 1 0 to 2 0 mg per 100 ml during the procedure In normal subjects there may be an increase in urine phosphorus to two to four times control values and serum phosphorus may fall by 0 5 to 1 mg per 100 ml [1 2] In patients with PHP the urine phosphorus characteristically shows no change or a rise to not more than twice control values and serum phosphorus does not change

The clinical application of this test presents four difficulties In the first place parathyroid extract may lose its potency To control this source of confusion the same batch of extract is often tested on a normal subject In the second place normal subjects may show little change in urine or serum phosphorus even with potent extract [21] This difficulty may be eliminated if it is possible to test the extract not on a normal subject but on a subject with known idiopathic hypoparathyroidism In the third place some subjects with pseudohypoparathyroidism well documented in all other respects may show a rise of urine phosphorus to as much as five times the control values [2 4] This type of response may be a result of nonspecific action of the extract on the kidneys [22 23] It is known that commercially available extracts quite consistently increase renal plasma flow and some of them increase glomerular filtration rate and thus filtered phosphorus as well [23 24] This third source of confusion is eliminated if glomerular filtration rate measured during the Ellsworth Howard test shows no significant rise Glomerular filtration rate has not generally been measured and hence many published data cannot be properly evaluated A fourth source of confusion arises from reports of patients with steatorrhea and no other signs of PHP who show an elevated serum phosphorus level [25] instead of the lowered serum phosphorus level generally seen with steatorrhea These patients may show no change of urine phosphorus during the Ellsworth Howard test [26] This difficulty may be eliminated when patients with this syndrome

are tested with parathyroid extract over a period of days as discussed below

In the first report of the syndrome of PHP all three subjects were shown to be resistant to parathyroid extract given intramuscularly over 3 to 17-day periods as judged from negligible changes in serum phosphorus and calcium Thus the confusion attendant upon nonspecific effects of the extract on renal function when administered intravenously was avoided and also the studies showed that in PHP there is resistance to the calcium raising property of parathyroid extract as well as to its ability to promote phosphate diuresis These findings strongly imply that the effect of parathyroid hormone on bone [27] as well as on the renal tubules is diminished or absent in PHP This conclusion derives also from the observation that most patients with PHP show normal to increased bone den ity normal serum alkaline phosphatase values and no signs of osteitis fibrosa cystica such as loss of lamina dura subperiosteal re orption or bone cysts If parathyroid hormone is indeed ecreted in excess in this syndrome osteitis fibrosa would be expected unless the resistance involves the bones as well as the kidneys

Resistance to parathyroid extract administered over a period of days has been used to aid in the diagnosis of PHI in only a few reported cases [1 2 4 28] In one case the initial re ponse to parathyroid extract given intravenously was normal but the longer test confirmed the clinical impression of I HP by producing little change in serum phosphorus and only a transient change in serum calcium [2]

The effects of parathyroid extract given intravenously or intramuscularly may be influenced by previous administration Acquired re istance to parathyroid extract has been well documented and may be associated with the presence of precipitating antibodies [29] Accordingly resistance to extract in a previously treated patient may give a false-positive diagnosis of PHI

The nature of the resistance to parathyroid hormone has not been clarified Two groups of workers [2 30] howed that the serum of patients with I HI would not interfere with the action of parathyroid extract given under standard conditions In one tudy [2] dogs howed a normal ri e in serum calcium with parathyroid extract given together with or previously incubated with serum from a patient with I HI

## METASTATIC OSSIFICATION

Patients with PHI characteri tically form bone in subcutaneous tissues and along fa cial planes This is true bone with normal hi tology There is no evidence of o teomalacia (wide osteoid eam ) and no evidence of osteitis fibro a (o teocla ts and fibrosis) Ectopic bone formation is the cardinal sign of myo itis os ificans progres iva a yndrome in which

shortness of metacarpal and metatarsal bones closely resembling that found in achondroplasia is also a regular feature. This has suggested [2] that a common genetic defect may relate PHP and myositis ossificans progressiva.

## ABNORMALITIES OF BONE

Abnormalities of metacarpal and metatarsal bones form a cardinal feature of PHP. These bones are characteristically short and thick. They may show abnormal curvature as well. The first, fourth, and fifth metacarpals are more frequently affected than the second and third [2]. There may or may not be early union of the epiphyses; the defect appears to be essentially a failure or limitation in linear growth at metaphyseal ends of bones exactly comparable to that seen in achondroplasia.

Exostoses have been noted in a number of cases [5–7]. These are indistinguishable from those seen in the syndrome of hereditary deforming chondrodysplasia [31] and, as in that syndrome, are frequently reported in the parents of patients with PHP. This finding lends support to the view that PHP represents a genetically determined defect.

## GENETIC ASPECTS

As noted above, PHP has a strong tendency to occur in more than one member of a family. This is a valuable aid to differential diagnosis, since familial incidence is almost unknown in idiopathic hypoparathyroidism, even though there are many more reported cases of that disorder than of PHP. In the three siblings with idiopathic hypoparathyroidism reported by Sutphin and associates [13], there was associated moniliasis in all cases, and all subjects also suffered from "familial Mediterranean target oval cell syndrome." In the family reported by Goldman and associates [32], hypocalcemia was found in three brothers who showed none of the other stigmata of PHP. The one patient who was studied showed a transient response to parathyroid extract given intravenously, when a single hourly urine phosphorus reached four times the control value. In the family reported by Lachman [10, Patients 57 to 59], the three siblings who were thought to have idiopathic hypoparathyroidism were "short, mentally sluggish and childish," and all had one or more short metacarpal bones. They were not tested for responsiveness to parathyroid extract, but it seems probable that they suffered from PHP. If PHP is a genetically determined disorder, and if it involves more than a single gene [2], one would expect to find incomplete cases. Although it is possible that the patients referred to above with steatorrhea, elevation of serum phosphorus level, and resistance to parathyroid extract [25, 26] represent the abnormality in parathyroid function alone, this hypothesis cannot be tested directly until the nature of the resistance in PHP is more fully understood.

## PSEUDO PSEUDOHYPOPARATHYROIDISM

One type of incomplete PHP has been well documented It is of interest that a number of reports have appeared de cribing cases with all the characteristic abnormalities of PHP except those associated with hypo parathyroidism [33-37] they have been given the name of pseudo p eudohypoparathyroidi m The abnormalities in the e patients include the short metacarpal and metatarsal bones with brachydactyly meta static ossification shortness of stature with roundness of the face mental deficiency and indeed lenticular opacities despite ab ence of hypocalcemia or of any history thereof These patients have normal erum calcium and phosphorus values and normal response to parathyroid extract They do not show the calcification of the basal ganglia char acteristic of idiopathic hypoparathyroidism and of PHP This suggests that this manifestation is a result of the chemical abnormalities and is not under separate genetic determination As in PHP familial incidence has been reported in pseudo-p eudohypoparathyroidism The presence of this incomplete syndrome lends strong support to the hypothesis that the fundamental disorder in PHP represents a genetically deter mined defect

Recently Van der Werff ten Bosch [38] has reported that four of six female patients with this syndrome had gonadal dysgene is The nuclear pattern of three of them was chromatin negative for genetic sex

## SUMMARY

1 Pseudohypoparathyroidism is a familial heritable disease in which the clinical and biochemical abnormalities of hypoparathyroidism are as ociated with skeletal abnormalities and metastatic o sification

2 Patients are characteristically short with round faces may have mental deficiency and show changes characteristic of achondroplasia in one or more metatarsal or metacarpal bones True ectopic bone is found in skin and in fascial planes and there may be calcification of the ba al ganglia

3 The chemical abnormalities include hypocalcemia and hyper phosphatemia Parathyroid extract produces little or no immediate pho phate diuresis and is ineffective in restoring serum calcium and pho phorus towards normal values Parathyroid tissue is normal or hyperpla tic The disorder repre ents not a deficiency of parathyroid hormone but re istance to its effects

4 Although only a ingle case has hown all the es ential features (characteri tic build metacarpal defect meta tatic o ification hypoparathyroidism resistance to parathyroid extract in hort term and long term courses and hyperpla ia of parathyroid ti ue) the syndrome seem to be a di tinct clinical entity

5 The syndrome of pseudo pseudohypoparathyroidism appears to represent the same entity except that hypoparathyroidism and resistance to parathyroid hormone are absent

## BIBLIOGRAPHY


1 Albright F Burnett, C H Smith P H and Parson W Pseudohypoparathyroidism—an example of Sebright Bantam syndrome Endocrinology 30 922 1942
2 Elrick, H Albright F Bartter F C Forbes A I and Reeves J O Further studies on pseudohypoparathyroidism report of four new cases Acta endocrinol 5 199 1950
3 Frame B and Carter S Pseudohypoparathyroidism Clinical picture and relation to convulsive seizures Neurology 5 297 1955
4 MacGregor M E and Whitehead T P Pseudohypoparathyroidism A description of three cases and a critical appraisal of earlier accounts of the disease Arch Dis Childhood 29 399 1954
5 Selye H Hypoparathyroidism in *Textbook of Endocrinology* pp 567–588 Université de Montreal 1947
6 Bishop P M F and DeMowbray R R. Pseudohypoparathyroidism Proc Roy Soc Med 44 952 1951
7 Mackler H Fouts J R and Birsner J W Familial pseudohypoparathyroidism A report of two cases Calif Med 77 332 1952
8 Uhlemann H J Familiare idiopathische Tetanie im rahmen pluriglandularer Insuffizienz Klin Wchnschr 28 489 1950
9 Azerad E Catha J and Raverdy I Un cas de pseudohypoparathyroidisme Bull et mém Soc méd hôp Paris 69 199 1953
10 Lachmann A Idiopathic hypoparathyroidism Acta med scandinav Suppl 121 1941
11 Bergstrand C G Ekengren K Filipsson R and Huggert A Pseudohypoparathyroidism Familial incidence and comparison with idiopathic hypoparathyroidism Acta endocrinol 29 201 1958
12 Buchs S Familiarer Pseudo-hypoparathyroidismus Ann paediat 183 65 1954
13 Sutphin A Albright F and McCune D J Five cases (three in siblings) of idiopathic hypoparathyroidism associated with moniliasis J Clin Endocrinol 3 625 1943
14 Gorceau G J and Miller W E Osteochondrodystrophy as a result of or in relation to pseudohypoparathyroidism J Bone & Joint Surg 38 A 131 1956
15 Albright F Hypoparathyroidism as a cause of osteomalacia J Clin Endocrinol 16 419 1956
16 Howat T W and Ashurst G M Pseudohypoparathyroidism J Bone & Joint Surg 39 B 39 1957
17 Butterworth C E Jr Hamilton L C and Zheutlin N Pseudohypoparathyroidism Am J Med 21 644 1956
18 Drake T Albright, F Bauer W and Castleman B Chronic idiopathic hypoparathyroidism report of six cases with autopsy findings in one Ann Int Med 12 1751 1939
19 Eaton L M Camp J D and Love J C Symmetric cerebral calcification particularly of the basal ganglia demonstrable roentgenographically Arch Neurol & Psychiat 41 921 1939
20 Ellsworth R and Howard J E Studies on the physiology of the parathyroid glands Bull Johns Hopkins Hosp 55 296 1934
21 Milne M O Observations on the action of the parathyroid hormone Clin Sc 10 471 1951
22 Stewart G S and Bowen H F The urinary phosphate excretion factor of parathyroid gland extracts a hormone or an artefact? Endocrinology 51 80 1952

23 Bartter F C The parathyroids Ann Rev Physiol 16 429 1954
24 Handler P and Cohn D V Use of radiophosphorus in studies of glomerular permeability of plasma inorganic phosphate Am J Physiol 164 646 1951
25 Bennett T I Hunter D and Vaughn J M Idiopathic steatorrhea (Gee s disease) Quart J Med 1 603 1932
26 Jackson W P U Hoffenberg R Linder G C and Irwin L Syndrome of steatorrhea pseudohypoparathyroidism and amenorrhea Observations on the calcium test and the effect of probenecid on calcium and phosphorus metabolism J Clin Endocrinol 16 1043 1956
27 Barnicot N A The local action of the parathyroid and other tissues on bone in intracerebral grafts J Anat 82 233 1948
28 Robinson I K Carmichael L A and Cummings J N Idiopathic hypoparathyroidism Quart J Med 23 383 1954
29 Lerman J Personal communication
30 Wise B L and Hart J C Idiopathic hypoparathyroidism and pseudohypoparathyroidism Observation on the electroencephalogram in hypocalcemia A M A Arch Neurol & Psychiat 68 78 1952
31 Bauer W and Bartter F C Hereditary deforming chondrodysplasia in *Textbook of Medicine* 10th ed edited by Russell L. Cecil and Robert F Loeb Saunders Philadelphia 1959
32 Goldman R Reynolds J L Cummings H R and Bassett S H Familial hypoparathyroidism J A M A 150 1104 1952
33 Albright F Forbes A P and Henneman P H Pseudo-pseudohypoparathyroidism Tr A Am Physicians 65 337 1952
34 Miles J and Elrick H Pseudo-pseudohypoparathyroidism report of a new case J Clin Endocrinol 15 576 1955
35 Wallach S Englert E Jr and Brown H The syndrome of pseudo pseudohypoparathyroidism A M A Arch Int Med 98 517 1956
36 Seringe P H and Tomkiewicz S Pseudo-pseudohypoparathyroidisme familial Ann endocrinol 17 655 1956
37 Roche M A case of pseudo-pseudohypoparathyroidism J Clin Endocrinol 15 964 1955
38 Van der Werff ten Bosch J J Brachymetatarsal dwarfism Lancet 1 69 1959

5 The syndrome of pseudo pseudohypoparathyroidism appears to represent the same entity, except that hypoparathyroidism and resistance to parathyroid hormone are absent

## BIBLIOGRAPHY


1 Albright F Burnett C H Smith P H and Parson W Pseudohypoparathyroidism—an example of Sebright Bantam syndrome Endocrinology 30 9 ? 1942
2 Elrick H Albright F, Bartter F C Forbes A P and Reeves J O Further studies on pseudohypoparathyroidism report of four new cases Acta endocrinol 5 199 1950
3 Frame B and Carter S Pseudohypoparathyroidism Clinical picture and relation to convulsive seizures Neurology 5 297 1955
4 MacGregor M E and Whitehead T P Pseudohypoparathyroidism A description of three cases and a critical appraisal of earlier accounts of the disease Arch Dis Childhood 29 339 1954
5 Selye H Hypoparathyroidism in *Textbook of Endocrinology* pp 56?–588 Université de Montreal 1947
6 Bishop P M F and DeMowbray R R Pseudohypoparathyroidism Proc Roy Soc Med 44 952 1951
7 Mackler H Fouts J R and Birsner J W Familial pseudohypoparathyroidism A report of two cases Calif Med 77 332 1952
8 Uhlemann H J Familiare idiopathische Tetanie im rahmen pluriglandularer *Insuffizienz Klin Wchnschr* 28 483 1950
9 Azerad E Gatha J and Raverdy P Un cas de pseudohypoparathyroïdisme Bull et mém Soc méd hôp Paris 69 139 1953
10 Lachmann A Idiopathic hypoparathyroidism Acta med scandinav Suppl 121 1941
11 Bergstrand C G Ekengren K Filipsson R and Huggert A Pseudohypoparathyroidism Familial incidence and comparison with idiopathic hypoparathyroidism Acta endocrinol 29 201 1958
12 Buchs S Familiärer Pseudo-hypoparathyroidismus Ann paediat 183 65 1954
13 Sutphin A Albright F and McCune D J Five cases (three in siblings) of idiopathic hypoparathyroidism associated with moniliasis J Clin Endocrinol 3 625 1943
14 Gorceau G J and Miller W E Osteochondrodystrophy as a result of or in relation to pseudohypoparathyroidism J Bone & Joint Surg 38 A 131 1956
15 Albright F Hypoparathyroidism as a cause of osteomalacia J Clin Endocrinol 16 419 1956
16 Howat T W and Ashurst G M Pseudohypoparathyroidism J Bone & Joint Surg 39 B 39 1957
17 Butterworth C E Jr Hamilton L C and Zheutlin N Pseudohypoparathyroidism Am J Med 21 614 1956
18 *Drake T Albright F Bauer W and Castleman B Chronic idiopathic hypoparathyroidism* report of six cases with autopsy findings in one Ann Int Med 12 1751 1939
19 Eaton L M Camp J D and Love J G Symmetric cerebral calcification particularly of the basal ganglia demonstrable roentgenographically Arch Neurol & Psychiat 41 921 1939
20 Ellsworth R and Howard J E Studies on the physiology of the parathyroid glands Bull Johns Hopkins Hosp 55 296 1934
21 Milne M O Observations on the action of the parathyroid hormone Clin Sc 10 471 1951
22 Stewart G S and Bowen H F The urinary phosphate excretion factor of parathyroid gland extracts a hormone or an artefact? Endocrinology 51 80 1952

Part Eight

# Diseases of Porphyrin Metabolism

# Chapter 30

# The Porphyrias *

*Rudi Schmid*

Since the end of the nineteenth century porphyria has aroused the curiosity and interest of clinicians and investigators alike and has received widespread attention in spite of its relatively infrequent occurrence. A continuing interest in disorders of porphyrin metabolism is largely due to two factors (1) the unusual variety of clinical manifestations associated with these disturbances and (2) the relative ease with which very small amounts of porphyrins can be identified and estimated owing to their unique spectroscopic and fluorescent properties.

Porphyria has been the subject of innumerable clinical reports and of many biochemical investigations but only relatively recently has information accumulated regarding the nature and location of the metabolic defects which may account for the appearance of the disease. In the last 12 years with the advent of tracer techniques impressive progress has been made and much of the biosynthetic pathway by which porphyrins and heme are formed has been clarified. This better understanding of the *normal* pathways of metabolism provides an indispensable basis for the elucidation of the biochemical defects involved in the various types of porphyria.

The first authentic case of porphyria described in the literature was reported by Baumstark in 1874 [1] under the diagnosis of "pemphigus leprosus". A craftsman with cutaneous photosensitivity and obvious liver disease was found to excrete a urinary pigment which was designated as "urorubrohaematin" and which had unmistakably the spectroscopic and solubility properties of the compound now known as uroporphyrin. A few years later MacMunn [2] reported on five patients suffering from "subacute rheumatism" or "idiopathic pericarditis" whose urine contained a red pigment which he named "urohaematin".

* This work was supported in part by grant A 1833(C) from the Public Health Service.

of clinical subforms with different manifestations such as abdominal colic peripheral neuropathy and paralysis psychosis and coma In addition patients who excreted abnormal amounts and types of porphyrins including porphobilinogen but in whom clinical manifestations of porphyria were lacking were classified as having *latent porphyria* [*14 16 18 19*] Such persons many of whom were blood relatives of patients with manifest porphyria were believed to exhibit the inherited metabolic abnormality but for unknown reasons they remained free of symptoms It was recognized however that in such asymptomatic patients acute clinical manifestations could at times be precipitated by ingestion of relatively small amounts of sedatives such as barbiturates and sulfonal [*14 20–22*] In these instances the drug undoubtedly converted a pre existing latent porphyria into an acute form [*14*]

On the other hand acute porphyria was reported with increasing frequency in individuals who were addicted to sedatives but in whom no evidence of preexisting porphyria or familial occurrence of the disease could be elucidated [*13 21 23 26*]

Soon after 1888 the year of the introduction of sulfonal and related compounds into clinical medicine [*25*] massive haematoporphyria was observed in two patients with chronic sulfonal poisoning [*26*] By 1900 Taylor [*27*] was able to collect from the literature 34 cases of fatal sulfonal poisoning most of these patients showed a marked increase in haematoporphyrin excretion in the urine Particularly striking was a patient with fatal porphyria described by Duesberg [*24*] who over a period of many years had ingested very large amounts of Sedormid (allyl isopropyl acetyl carbamide) and other sedatives His urine exhibited high concentrations of uroporphyrin and at autopsy intense porphyrin fluorescence was demonstrable in the liver gallbladder and bile ducts [*28*]

The concept that in previously normal individuals sedatives of the sulfonal group could produce a porphyria like syndrome gained strong support from animal experiments In rabbits it was observed that sulfonal poisoning regularly produced marked haematoporphyrinuria [*6 25 29–31*] With more refined analytic methods Fischer and Duesberg [*32*] demonstrated uroporphyrin in the urine of such rabbits but a few years later Waldenstrom and Wendt [*33*] failed to confirm these observations The question remained undecided until 1952 when it was discovered that in rabbits the sedative Sedormid also produced a syndrome which exhibited many biochemical similarities to human hepatic porphyria [*34*] On a weight basis these animals were found to excrete more porphobilinogen and porphyrins than porphyric patients in relapse Nevertheless certain biochemical differences between the hereditary human disorders and experimental Sedormid porphyria in animals were recognized In the latter condition there was a precipitous fall in liver catalase activity

Although "urohaematin" undoubtedly was a porphyrin it is questionable whether MacMunn was actually describing uroporphyrin Its solubility in chloroform and its spectroscopic properties which differed slightly from Baumstark's "urorubrohaematin" [3] suggest that MacMunn may have been dealing with the pigment which is today designated as coproporphyrin In spite of the recognized slight spectroscopic differences Salkowski [3] Hammersten [4] Garrod [5] Stokvis [6] Saillet [7] Anderson [8] Nebelthau [9] and others regarded these pigments for practical purposes as identical with hematoporphyrin [1] which Hoppe-Seyler [10] and Nencki and Sieber [11] had prepared from hemoglobin by the action of concentrated sulfuric acid Garrod [5] discovered "haematoporphyrin or allied pigments" in the urine of 76 out of a group of 176 patients of a mixed hospital population His conclusion that "haematoporphyrin" was an "almost constant constituent of urine in health and disease" reached far ahead into modern concepts of porphyrin metabolism During the next two decades massive "haematoporphyrinuria" [1] was observed in a large number of patients exhibiting such diversified clinical manifestations as photodermatitis colicky abdominal pain bizarre involvement of the nervous system or psychic disturbances

## PROPOSED CLASSIFICATIONS

Guenther [12] was the first in attempting to arrange the reported cases according to certain recurrent symptom patterns His classification distinguished between *haematoporphyria congenita* and *haematoporphyria acuta* the latter including idiopathic and toxic forms In addition he designated a few cases as *haematoporphyria chronica* a group which he originally considered as a separate entity but later tended to regard as mild and late forms of *porphyria congenita* [13] In such patients the photosensitivity and the excretion of red urine are indeed characteristic of what is now termed congenital (erythropoietic) porphyria yet these two conditions differ strikingly in their clinical course and particularly in the time of onset of the first symptoms In 1937 Waldenstrom [14] proposed to substitute the term *porphyria cutanea tarda* for Guenther's original "chronic porphyria" In recent years the fundamental difference between *congenital photosensitive porphyria* as a defect in the erythropoietic system and *porphyria cutanea tarda* as a disturbance in liver metabolism has become well recognized [15–17]

Under the term *acute porphyria* Waldenstrom [14] included a variety

[1] Hematoporphyrin is not found in nature but can be prepared from hemoglobin in vitro In the earlier literature hematoporphyrin was used as a generic term to designate all naturally occurring porphyrins as well as those which can be derived from hemoglobin in vitro Hematoporphyria hematoporphyrinuria and porphyria were terms employed interchangeably

From the above discussion it is evident that it may at times be difficult if not impossible to decide whether a patient is suffering from an inherited disorder or from an acquired disturbance of porphyrin metabolism. This is more frequently the case in porphyric patients exhibiting photosensitivity in whom liver disease is a prominent feature but similar difficulties may be encountered in patients with acute symptoms who have used excessive amounts of sedatives. The problem is further complicated by the fact that increased excretion of porphobilinogen and of uroporphyrins has been observed in patients with hepatic neoplastic and neurologic diseases in whom symptomatic manifestations of porphyria were absent [44, 45].

The following discussion will be limited to those forms of porphyria for which a hereditary basis has clearly been established. For a more general consideration of disturbances in porphyrin metabolism the reader is referred to recent reviews by Watson [39], Rimington [46], Waldenström [16], Vannotti [47], and Bénard [48].

## RELATIONSHIP OF PORPHYRINS TO HEMOGLOBIN METABOLISM

The name *porphyrin* appeared for the first time in the literature in 1871 when Hoppe Seyler [10] prepared haematoporphyrin from hemoglobin. A few years later Nencki and Sieber [11] identified haematoporphyrin as a derivative of hemin, the prosthetic group of hemoglobin. Although a number of investigators [2, 3, 5, 7, 31] recognized the slight spectroscopic differences between hematoporphyrin and the porphyrins excreted in the urine, for practical purposes these pigments were considered as identical. This led to the widely accepted but erroneous belief that the urinary porphyrins are derived from breakdown of hemoglobin [?, 6, 13, 47, 49–51], although several observers including Kast and Weiss [31], Garrod [5], and later Liebig [52] raised doubts as to the validity of this concept. It was not until 1925 that direct proof became available for the independence of porphyrin excretion from hemoglobin breakdown. Fischer and his coworkers [53] found significant structural differences between hematoporphyrin and the porphyrins present in the urine. More important still was the finding that the urinary porphyrins belong in part to an isomeric series different from that of hemoglobin protoporphyrin (Fig 30-1).* These and other observations led Fischer to sug

* The classification of porphyrin isomers is based on the four synthetically prepared isomers of etioporphyrin designated as I, II, III, and IV. In nature only prophyrins belonging to isomer series I and III are found. The protoporphyrin of hemoglobin and of other heme proteins has the basic structure of a porphyrin of type III isomer. However, since the $\beta$ positions of the pyrrole rings are substituted by three different radicals, the total number of possible isomers for protoporphyrin is 15. The protoporphyrin occurring in all known heme proteins is designated as isomer 9a and corresponds to the basic structure of etioporphyrin III.

[35] the urinary porphyrins were almost exclusively of the type III isomer [34] and the concentrations of proto and coproporphyrin in the liver and bile were greatly increased [36] On the other hand, no increase in the urinary excretion of δ aminolevulinic acid was observed [37] although porphobilinogen was present in high concentrations [36]

These animal experiments support the concept that in persons who habitually ingest large amounts of sedatives, a *toxic* form of porphyria may develop This is distinct from latent *hereditary* porphyria in which relatively small amounts of sedatives may precipitate an acute attack Since detailed studies of human toxic porphyria have not been reported it is not known how this condition differs from the hereditary forms of porphyria The chemical findings in experimental Sedormid porphyria suggest that the metabolic derangement in toxic porphyria may be quite different from that in the genetically controlled disorders

The concept that some forms of human porphyria may not have a hereditary basis has recently gained additional support from studies of patients with cutaneous porphyria The term "porphyria cutanea tarda symptomatica has been proposed by Waldenstrom [16] to describe patients in whom outspoken liver disease is associated with photosensitivity and marked elevation of porphyrin excretion In this group he included some of the patients who previously had been reported as having porphyria cutanea tarda by Brunsting [38] Watson [39] Bolgert et al [40] Schmid et al [41] and Barnes [42] and in whom liver disease was a prominent feature Waldenstrom believes that in such patients the liver disease usually of alcoholic or nutritional origin is the primary disorder and that the disturbance in porphyrin metabolism is merely a secondary manifestation [16] An interesting case of seemingly acquired cutaneous porphyria was recently reported by Tio Tiong Hoo [43] In an 80-year-old woman photosensitivity and marked porphyrinuria had gradually developed over the course of a year, when she was discovered to have a large benign liver cell adenoma On resection this was found to contain large amounts of proto- copro- and uroporphyrin the surrounding liver tissue was normal Following the operation the cutaneous manifestations disappeared and the porphyrin excretion returned to normal values This seemed to indicate that the defect in porphyrin metabolism was limited to the hepatic tumor

Although in most of the above-discussed patients there is no positive family history nor evidence suggesting a preexisting latent porphyria the possibility cannot be excluded with certainty that a predisposition to porphyria may have been inherited and the acquired liver disease may merely have served to bring out the clinical and chemical manifestations of this disturbance Moreover it should be noted that several genetically distinct forms of porphyria exhibiting cutaneous manifestations have been described in which the hereditary nature of the disorder has clearly been established These will be discussed later

zymes such as cytochromes catala e and peroxidase [60] Although the amounts of these heme proteins in the organism may be quite small as compared with hemoglobin some of them may have a more rapid turn over than hemoglobin For liver catalase for example the half life has been estimated to be only a few days [35 61] a rate of turnover which requires the daily synthesis of a considerable amount of protoporphyrin It is therefore conceivable that metabolic abnormalities resulting in excessive formation and liberation of porphyrins and of porphyrin precursors may occur in any tis ue of the body but that the liver is of particular importance as the site of such disturbances In fact with the exception of congenital erythropoietic porphyria the liver seems to be the major site of the metabolic derangement in most forms of human porphyria and in experimental porphyria produced by Sedormid This has led to the proposal to classify these disturbances under the group name of *hepatic porphyria* [18 39 41]

## BIOSYNTHESIS OF PORPHYRINS AND OF HEME

Little was known prior to 1946 about the chemical building blocks from which the body synthesizes porphyrins and heme In that year Shemin and Rittenberg [62 63] observed that after feeding isotopically labeled glycine to man or animals the concentration of the label in the circulating heme of hemoglobin rose rapidly remained more or less

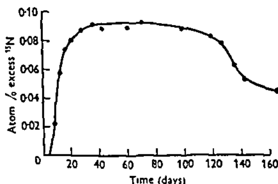



Fig 30- Con entration of N[15] in hemoglobin heme of a normal subje t given 12 gm glyc n N (31 f5 atom per cent exce s N ) during the first 4 days of the experiment (*By per sion of C H Gray* [ 30] )

steady for about 100 days and then rapidly declined (Fig 30 2) The disappearance of the label from the circulating heme was accompanied by a marked though temporary increa e in the fecal excretion of labeled urobilinogen [64 65] (Figs 30 2 30 14) These ob ervations permitted the conclusions that (1) glycine is a pecific precursor of heme protopor

gest that in congenital (erythropoietic) porphyria porphyrins of the type I isomer are synthesized along with and parallel to hemoglobin formation in developing erythoid cells of the bone marrow Fischer considered this "atavism as a partial regression into a phylogenetically and ontogenetically more primitive phase of development As will be seen later this concept is to a large extent in agreement with more recent studies

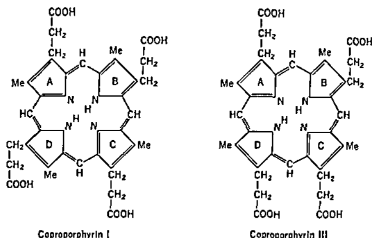



Fig 30-1 Coproporphyrin of type I and type III isomer series In porphyrins of type III isomer the sequence of the side chains in the $\beta$-positions of pyrrole ring D is reversed *Me* = methyl

Although as a result of these observations it was generally accepted that porphyrins may be formed along with hemoglobin in the bone marrow it soon became apparent that this hypothesis was not applicable to patients with acute porphyria and porphyria cutanea tarda On the basis of extensive clinical and necropsy studies Waldenstrom [*64*] came to the conclusion that there must exist a fundamental difference in the pathogenesis of the various forms of porphyria He reasoned that ' the porphyrin must either be an anomalous product on the way to hemoglobin which is then excreted (or) the other possibility is to try to find another source from which porphyrin might appear The nature of this other source was suggested by autopsy studies [*28 35 41 47 65–69*] and by analysis of biopsy material obtained from patients with porphyria [*41*] These investigations were supplemented by animal experiments as discussed in the preceding section

As a result of these observations it became increasingly clear that not only the erythropoietic system but also other organs and particularly the liver may be important sites of porphyrin formation This is not surprising since most mammalian cells can synthesize the porphyrins which are required for the formation of essential heme-containing en

[70 75] With methyl labeled acetate for example highest labeling of heme protoporphyrin is observed in the carbon atoms 6 and 9 (Fig 30-4) less activity is present in positions 4 8 5 and 3 [76] Moreover there is equal labeling each to each in the position pairs 6 and 9 4 and 8 5 and 3 [76] The labeling pattern obtained in protoporphyrin synthesized from succinate 1 4 $C^{14}$ is pictured in Fig 30-4 [79]

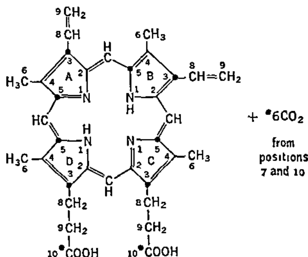



Fig 30-4 The labeling pattern obtained in protoporphyrin synthesized from succinate 1 4-C Carbon which gives rise to the CO is the carbon of the original carboxyl group attached to carbon 6 (*By permission of D Shemin [70]*)

These findings indicate that the biosynthesis of the protoporphyrin molecule requires 8 moles of glycine and 8 moles of a four-carbon intermediate of the tricarboxylic acid cycle most likely succinate [70 74] The possible ways by which succinate and glycine could combine to form a pyrrole unit are limited by the finding that the α-carbon atom of glycine is always *equally* utilized for both the pyrrole ring carbon atoms *and* the methene bridge carbon atoms [70 77]

The above and other observations led Shemin and his coworkers [70 78] to suggest that the intermediate which is formed by condensation of glycine and succinate is the five carbon aminoketone δ aminolevulinic acid (ALA) (Fig 30 3) With the use of ALA 5 $C^{14}$ in a system of hemolyzed duck red cells the position of this aminoketone as a true intermediate in porphyrin biosynthesis has been well documented [79-81] Not only was the labeling pattern of the δ carbon atom of ALA identical with that of the α-carbon atom of glycine [70 79] (Fig 30-3) but the heme synthesized from an equimolar amount of ALA 5 $C^{14}$ was about sixty five times more radioactive than heme synthesized from glycine

phyrin, (2) hemoglobin of the circulating erythrocytes remains outside the general metabolic pool of protein interchange (3) the majority of normal red cells have a mean life span of about 120 days (4) a large fraction of the fecal urobilinogen is derived from the breakdown of circulating red blood cells, and (5) the metabolites of heme protoporphyrin are not reutilized for the synthesis of hemoglobin in newly formed erythrocytes

Additional studies carried out in various laboratories showed that blood from ducks and chickens which contains nucleated erythrocytes [66

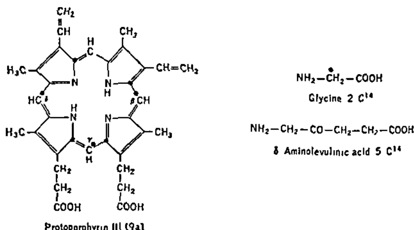



Fig 30-3 The carbon atoms of protoporphyrin derived from the α-carbon of glycine and the δ-carbon of δ aminolevulinic acid (ALA) (*By permission of D Shemin [ 0]*)

67] and blood from rabbits which had been made anemic to produce a high percentage of reticulocytes [68] is able to synthesize labeled heme from glycine $C^{14}$ in vitro The availability of these systems greatly facilitated subsequent studies which revealed that all four nitrogen atoms [62 69 70] and eight carbon atoms of the protoporphyrin molecule are derived from glycine [70–72] (Fig 30 3) Four of the carbon atoms are still attached to the nitrogen atoms of glycine while four are not forming the methene bridges which link the four pyrrole rings to form the porphyrin ring (Fig 30 3) All eight carbon atoms are derived from the α carbon atom of glycine while the carboxyl carbon of glycine is not utilized for any of the carbon atoms of porphyrin [72 73]

The remaining 26 carbon atoms of protoporphyrin arise from intermediates of the tricarboxylic acid cycle [70 74] Acetate labeled either in the methyl or in the carboxyl group α ketoglutarate-5 $C^{14}$ α keto glutarate-1 2 $C^{14}$ or citrate 1 5 $C^{14}$ lead to characteristic and predictable labeling patterns which strongly suggest that the same four-carbon chain is utilized in the formation of both sides of all four pyrrole rings

reactions all involving the transformation of glycine [70] The formation of porphyrins is only one of these various metabolic pathways

### BIOSYNTHESIS OF δ AMINOLEVULINIC ACID (ALA)

The elucidation of the exact mechanism by which ALA is formed by condensation of glycine with active succinate was a difficult task Shemin s observations indicated that the system required some particulate matter present in nucleated red cells of birds or in reticulocytes of mammals [70 78 79] but absent from mature mammalian erythrocytes [68] The e findings have now been confirmed and extended [83–86] but the nature

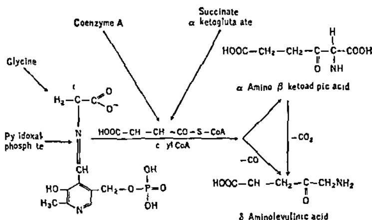



Fig 30-6 Condensation of succinyl CoA with glycine in the presence of pyridoxal phosphate (*Adapted from Kikuchi et al* [87])

of the cellular particles has not been conclusively established [85] (Fig 30-6) In more recent studies Kikuchi et al [87] Gibson [88] and Sawyer and Smith [89] have observed enzymatic synthesis of ALA from succinyl CoA in particle-*free* extracts obtained from *Rhodopseudomonas spheroides* or *Rhodospirillum rubrum* A forty to eighty fold purification of the soluble enzyme has been achieved [87]

Shemin and Wittenberg s original suggestion that the ' active four carbon intermediate may be succinyl CoA [74 76 77] has gained strong though indirect support from studies of heme and porphyrin formation in pantothenate deficient ducks [90] and in *Tetrahymena vorax* [91] The more recent studies by Gibson Laver and Neuberger [84] Shemin et al [87] Granick [85] and Brown [92] have provided conclusive evidence that succinyl CoA is indeed the intermediate which condenses with glycine The succinyl CoA can be derived from α ketoglutarate by the action of α ketoglutaric dehydrogenase [83 84] or it may be formed from succinate in the presence of ATP [84 87 92] (Fig 30 6)

2 $C^{14}$ [70] From these observations Shemin [78] postulated a metabolic pathway by which "active" succinate can be condensed with glycine to form the pyrrole precursor (Fig 30-5)

The existence of a succinate-glycine cycle which is interrelated with the tricarboxylic acid cycle was established by the finding that α amino

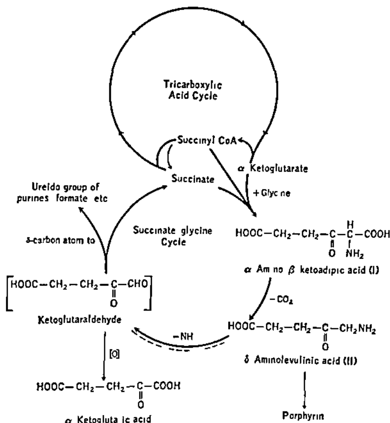



Fig 30-5 The succinate-glycine cycle Condensation of succinyl and glycine results in α amino-β ketoadipic acid (I) which is decarboxylated to δ-aminolevulinic acid (II) II is drawn off for porphyrin synthesis or oxidized to ketoglutaraldehyde which is decarboxylated to succinate (*By permission of D Shemin [70]*)

β ketoadipic acid can replace ALA as an intermediate in porphyrin biosynthesis [70] Moreover in addition to porphyrin the δ carbon atom of ALA like the α carbon atom of glycine labels the ureido group of purines the β carbon of serine and the methyl groups of methionine [70] (Fig 30 5) Furthermore if a cell free extract of duck erythrocytes is incubated with ALA 5 $C^{14}$ radioactive α ketoglutarate can be isolated [70] if in the same system ALA 1 4-$C^{14}$ is the substrate labeled $CO_2$ is obtained [82] Thus ALA appears to be an important intermediate in several

first by Westall [102] The soluble enzyme ALA dehydrase has been purified from a number of sources including duck erythrocytes [98] chicken erythrocytes [103] ox liver [99 100] and spinach leaves [104] Reduced glutathione is required for activation of the enzyme system [98 100 105] SH group inhibitors such as iodoacetamide and p chloromercuribenzoate have an inhibitory effect [100] The latter can be overcome by the addition of reduced glutathione [99 100] In addition marked inhibition of ALA dehydrase has been noted with the chelating agent ethylenediaminetetraacetic acid a finding which suggests that a metal may be essential in this enzymatic step This has recently been

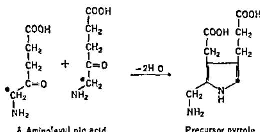



Fig 30-7 Condensation of 2 moles of δ aminolevulinic acid to form 1 mole of precursor pyrrole (porphobilinogen) The carbon atoms bearing the label are the original α-carbon atoms of glycine (*By permission of D Shemin [ 0]*)

confirmed by the observation that ALA dehydrase contains copper [106] Furthermore ducklings deficient in copper were found to exhibit reduced ALA dehydrase activity in liver and erythrocytes [106] Similar observations have been reported in copper-deficient chicks [107] and copper deficiency has been shown to lead to hypochromic anemia

Two moles of ALA are required for formation of 1 mole of PBG [70 98] (Fig 30 7) Heme synthesized from labeled ALA has twice the specific activity of that which is formed from an equimolar amount of PBG with the same radioactivity [70 98] These findings confirmed the earlier reports by Falk Dresel and Rimington [108] and by Bogorad and Granick [109] that PBG is a specific precursor and an obligatory intermediate in the biosynthesis of porphyrin and of heme

## BIOSYNTHESIS OF UROPORPHYRINOGEN

In considering the conversion of PBG to porphyrins and to heme two important findings have to be taken into account In earlier studies it had been assumed that simple linear condensation of four pyrrole units would lead to formation of the next metabolic intermediate uroporphyrin the latter would then undergo stepwise decarboxylation to yield protoporphyrin It soon was discovered however that this simple scheme was not correct In a variety of systems which were able to convert PBG to

The condensation of glycine with succinyl CoA requires the presence of pyridoxal phosphate. The requirement for pyridoxal was demonstrated along three different lines of evidence. In blood of pyridoxine-deficient ducklings Schulman and Richert [90] observed reduced heme synthesis from glycine $C^{14}$ a defect that could be corrected by the addition of pyridoxal phosphate. This finding is in line with earlier observations by Wintrobe [93] that pigs deficient in vitamin $B_6$ produced small pale red cells with a low content of hemoglobin and of free protoporphyrin. In vitro compounds known to inhibit enzymes which contain pyridoxal phosphate [94] have been shown to reduce the formation of ALA [83, 84, 87] and of porphyrins [85, 95]. Marked inhibition was obtained with L-penicillamine, isonicotinic acid hydrazide, cyanide, L-cysteine and p-chloromercuribenzoate. Finally biosynthesis of ALA [83, 84, 87, 9?] and of porphyrin [85, 90, 91] was found to be enhanced by the addition of pyridoxal phosphate in vitro. These findings indicate that pyridoxal phosphate is essential for the condensation of glycine with succinyl CoA and it appears likely that the cofactor is firmly bound to the condensing enzyme (Fig. 30-6) [83, 84, 87].

The primary condensation product is probably α amino β ketoadipic acid [70, 78, 80] (Figs. 30-5, 30-6), but the ease with which this compound undergoes spontaneous decarboxylation has prevented its isolation [84]. However, the finding that in the intact rat injection of the diethyl ester of this aminoketone resulted in urinary excretion of porphobilinogen provides at least indirect evidence to support this hypothesis [150].

The biosynthetic pathway of ALA formation is summarized in Fig. 30-6. Aerobic reactions which are inhibited by dinitrophenol [85, 96] convert α ketoglutarate [83, 84] or succinate [87, 92] to succinyl CoA. An enzyme which requires pyridoxal phosphate as a cofactor catalyzes the condensation of succinyl CoA with glycine to form α amino β keto adipic acid. By decarboxylation the latter is converted to ALA. The initial reaction of this pathway requires a functioning tricarboxylic acid cycle and thus is oxygen dependent; the condensing enzyme system is operative under anaerobic conditions. In mature mammalian erythrocytes the absence of a functioning tricarboxylic acid cycle obviously precludes formation of succinyl CoA and hence of ALA.

## BIOSYNTHESIS OF PORPHOBILINOGEN (PBG)

With the establishment of ALA as an obligatory intermediate in porphyrin and heme biosynthesis, studies of the subsequent enzymatic steps were greatly facilitated. ALA dehydrase was found to catalyze condensation of 2 moles of ALA to form a precursor pyrrole [97–100] (Fig. 30-7). The theoretical formulation of the structure of this pyrrole [78] yielded the same structure as porphobilinogen (PBG) [101], a chromogen excreted in the urine of patients with acute porphyria [14] and isolated

type III isomer is as yet incomplete sufficient information is available to formulate a reasonable working hypothesis [113] (Fig 30 9) Incubation of PBG with an enzyme system obtained from *Chlorella* [109] or from red cell hemolysates [124 125] results in formation of porphyrins of type III isomer However if such a system is heated for 15 min at 55 C it is changed in such a way that only porphyrins of type I isomer are formed This finding suggests that the enzyme porphobilinogenase may have more than one component Indeed Bogorad [116 117] and Hoare and Heath [114] succeeded in preparing two separate enzyme fractions PBG deaminase and uroporphyrinogen isomerase It appears that one of the actions of PBG deaminase is to remove the amino group and to condense individual monopyrroles to form polypyrrylmethanes (Fig 30-9) [105 117] The enzyme is relatively heat stable and if acting alone converts PBG to uroporphyrinogen I [116] Inhibition has been observed with silver and mercury ions and with formaldehyde [116] The latter may be of biologic importance as a possible regulatory mechanism promoting formation of uroporphyrinogen III since formaldehyde is formed in the deamination of the aminomethyl group of PBG [126]

If uroporphyrinogen isomerase from wheat germ is added to a system containing PBG and PBG deaminase the reaction product is uroporphyrinogen III [117] (Fig 30-9) Since by itself uroporphyrinogen isomerase reacts with neither PBG nor uroporphyrinogen I [113 117] it is clear that its enzymatic function is dependent on some effect of PBG deaminase on PBG It seems reasonable to assume that the isomerase acts by combining PBG with a noncyclized intermediate which is produced by the action of PBG deaminase on PBG [117] Such an intermediate may be a di or tripyrrylmethane [105 117] (Fig 30 9)

## METABOLISM OF UROPORPHYRINOGEN

The further metabolism of uroporphyrinogen can proceed along two separate lines it may undergo either a stepwise oxidation to uroporphyrin or a stepwise enzymatic decarboxylation to porphyrinogens which exhibit less than eight carboxyl groups [112–114 118 124] (Fig 30-10) The oxidative process leads to uroporphyrin which is a side product and does not serve as a substrate for the enzyme uroporphyrinogen decarboxylase Nonenzymatic oxidation of uroporphyrinogen can occur by photocatalytic autoxidation a process activated by the product uroporphyrin (Fig 30 10) [105] *Photooxidation is partially inhibited by* glutathione 2 mercaptoethylamine cysteine or sodium sulfite and is markedly reduced under strictly anaerobic conditions or by exclusion of light [105 116] On the other hand ferric ion at acid pH catalyzes the photooxidative process [105]

The conversion of uroporphyrinogen to uroporphyrin proceeds stepwise with formation of cyclized tetrapyrroles which exhibit an oxidation

heme or to porphyrins with less than eight carboxyl groups, uroporphyrin was ineffective as a substrate and could not replace PBG [70 110 111] These observations suggested that the true intermediate formed from PBG is not uroporphyrin but its reduced form uroporphyrinogen Indeed Neve and his coworkers [112] found that in a hemolyzed red cell system incorporation of $Fe^{59}$ into heme was markedly stimulated by the addition of uroporphyrinogen but not by uroporphyrin Additional studies [104 113-118] have clearly indicated that the biosynthetic path way from PBG to heme proceeds over a series of porphyrinogens which are colorless reduced porphyrins containing six additional hydrogen

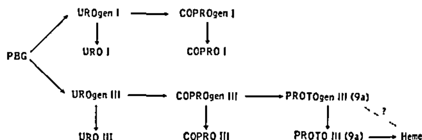



*Fig 30-8 Metabolism of porphobilinogen Only porphyrinogens of type III isomer* are intermediates in the biosynthesis of heme Porphyrins of type III isomer and porphyrinogens and porphyrins of type I isomer are side products not utilized for heme synthesis PBC porphobilinogen UROgen COPROgen PROTOgen uroporphyrinogen coproporphyrinogen protoporphyrinogen URO COPRO PROTO uroporphyrin coproporphyrin protoporphyrin

atoms [119] The oxidized porphyrins instead of being true intermediates are merely by products resulting from irreversible oxidation of the corresponding porphyrinogens (Fig 30 8) [120]

A second point of importance is that the protoporphyrin of hemoglobin and of other heme containing proteins belongs to the type III isomer series (protoporphyrin 9a) [53] (Fig 30 1) Heme proteins containing a porphyrin of type I isomer have never been found in nature [121] Thus, any biosynthetic scheme proposed for the formation of heme must include a mechanism which permits synthesis of type III isomer porphyrins Since in nature isomerization of porphyrins by ring opening and reclosure in a different position does not appear to occur [113] the isomer type formed must be determined at the time of the original ring closure (Fig 30-1) The exact mechanism by which this occurs is still in doubt Several suggestions have been advanced [70 105 109 122] but they will not be discussed in detail It may suffice to state that PBG can condense to uroporphyrin by nonenzymatic means and that under such conditions mixtures of uroporphyrin isomers are obtained [122 123]

Although knowledge regarding the precise mechanism and nature of some of the intermediates involved in the formation of porphyrins of

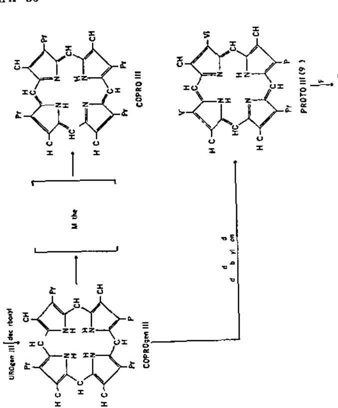



Fig 30-9 Scheme of heme biosynthesis from porphobilinogen Ac —CH COOH Pr —CH CH COOH Vi —CH=CH$_2$ UROgen COPROgen uroporphyrinogen coproporphyrinogen URO COPRO PROTO uroporphyrin coproporphyrin protoporphyrin (By permission of S Granick and D Mauzerall [105])

level intermediate between uroporphyrinogen and uroporphyrin [*113 114 116*] Mauzerall and Granick have suggested that these intermediates may be porphomethene and porphodimethene both of which show strong absorption at 500 mμ [*113*] The former has two and the latter four hydrogen atoms less than uroporphyrinogen (Fig 30-10) These partially oxidized intermediates may be obtained by slow oxidation of uropor phyrinogen with iodine in the dark or by reduction of uroporphyrin with sodium amalgam [*113 119*] Enzymatic oxidation of uroporphyrinogen catalyzed by a heat labile oxidase obtained from spinach leaves [*116*] appears to proceed along similar lines The oxidase can be separated from PBG deaminase by starch block electrophoresis and is markedly inhibited by hydroxylamine [*116*] Its specificity is not known and it appears to be of little biologic significance The same holds true for non enzymatic oxidation of uroporphyrinogen Since the amount of uro porphyrin normally excreted in the urine is exceedingly small as compared with the magnitude of heme synthesis the fraction of uroporphyrinogen that is oxidized and escapes from the biosynthetic path must be in

See page 955 for legend

degree of specificity ALA PBG and uroporphyrin cannot replace uroporphyrinogen as substrates [113 118] Mercury copper manganese and oxygen strongly inhibit the reaction [113 118] the latter probably by enhancing autoxidation of the substrate to uroporphyrin On the other hand reduced glutathione and cysteine greatly increase the yield of coproporphyrinogen A heat stable ultrafiltrable cofactor has been described in *R spheroides* [114] which appears to be essential for the enzymatic decarboxylation of uroporphyrinogen The isomer specificity of uroporphyrinogen decarboxylase is not known Both uroporphyrinogen I and III can serve as substrates [113 118] but the rate of decarboxylation appears to be higher for the type III isomer [113]

The removal of the four acetic acid side chains of uroporphyrinogen probably occurs stepwise and randomly so that intermediate porphyrinogens with seven six and five carboxyl groups are formed [104 113 120] Since the decarboxylating enzyme(s) appears to have a low Michaelis constant and a high turnover number [113] the concentration of the e intermediate porphyrinogens at any one given time is very low and the reaction is rapidly carried through to coproporphyrinogen As for uroporphyrinogen photocatalytic autoxidation of porphyrinogens with less than eight carboxyl groups is probably of little biologic importance since exclusion of light and the presence of antioxidants in the cells maintain them in a reduced state Under aerobic conditions in vitro however porphyrins with seven to five carboxyl groups are readily obtained [103 104 113] It is not known whether the successive removal of carboxyl groups of uroporphyrinogen to form ultimately coproporphyrinogen is catalyzed by a single or by several closely related enzyme systems [113] For convenience the term uroporphyrinogen decarboxylase is used although the enzyme as defined can react with porphyrinogens which exhibit fewer carboxyl groups than uroporphyrinogen

With uroporphyrinogen I as substrate coproporphyrinogen I or its oxidation product coproporphyrin I is the end product since further decarboxylation of the type I isomer series does not occur [113 118 120 128] Protoporphyrin or heme corresponding to type I i omer has never been demonstrated in nature [121 128] With uroporphyrinogen III on the other hand enzymatic decarboxylation may yield porphyrins of type III isomer with less than four carboxyl groups [118 129] Granick and Mauzerall [129] have presented evidence which strongly suggests that an enzyme bound to insoluble components of chicken erythrocytes or of *Euglena* is involved in the oxidative decarboxylation of coproporphyrinogen III to form the dicarboxylic protoporphyrinogen III The latter through intermediate stages of autoxidation may be converted to protoporphyrin III (9a) [10 129] The requirement for a particulate enzyme system for oxidative decarboxylation of coproporphyrinogen III has also been demonstrated by the findings of Rimington and Booij

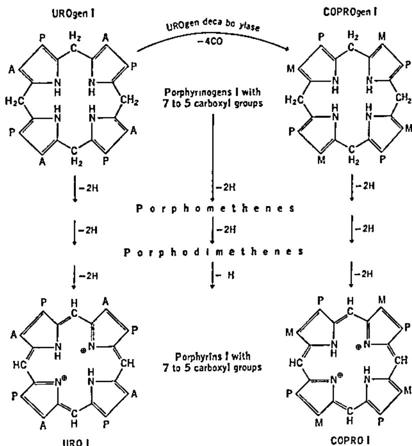



Fig 30-10 Stepwise conversion of uroporphyrinogen to coproporphyrinogen by uroporphyrinogen decarboxylase with formation of intermediate porphyrinogens with 7 6 and 5 carboxyl groups Each porphyrinogen can undergo stepwise oxidation to its respective porphomethene porphodimethene and finally porphyrin (For abbreviations see Fig 30-8)

significant It appears likely that the exclusion of light and the presence in the cells of antioxidants such as glutathione and cysteine are of importance in keeping most of the tetrapyrroles in a reduced state [*113*]

The major fraction of the uroporphyrinogen is enzymatically decarboxylated to porphyrinogens with less than eight carboxyl groups [*103 113 114, 118 124*] (Fig 30-10) Uroporphyrinogen decarboxylase activity has been demonstrated in erythrocytes of man [*124 125*] ducks [*112*] and chickens [*127*] in rabbit reticulocytes [*113*] in *Chlorella* [*109*] and in *Rhodopseudomonas spheroides* [*114*] With enzyme preparations obtained from rabbit reticulocytes [*113*] or *Chlorella* [*118*] anaerobic incubation of uroporphyrinogen resulted in formation of coproporphyrinogen exhibiting four carboxyl groups The enzyme has a high

of succinyl CoA pyridoxal phosphate for the condensation of succinyl CoA with glycine and copper as part of ALA dehydrase It appears likely that other cofactors may also be required but their nature has not yet been recognized [114]

4 ALA and PBG are obligatory intermediates in the biosynthesis of porphyrins and of heme

5 Porphyrinogens and porphyrins of the type I isomer series are by products of heme synthesis without known physiologic function In vivo the biosynthetic pathway is conditioned in such a way that formation of porphyrins of type I isomer is insignificant as compared with the amount of heme formed

6 With the probable exception of protoporphyrin III (9a) porphyrins of the type III isomer series are *not* intermediates in heme biosynthesis but are by products resulting from *irreversible* oxidation of the respective porphyrinogens The *true* intermediates are porphyrinogens of the type III isomer which are reduced porphyrins containing an additional six hydrogen atoms Conversion of porphyrinogens to porphyrins occurs by photocatalytic autoxidation or by the enzyme porphyrinogen oxidase In vivo oxidation of porphyrinogens to porphyrins is minimized by the exclusion of light and by the presence of antioxidants

7 The dynamics of the uroporphyrinogen decarboxylase system favor the formation of porphyrins with eight carboxyl groups (uroporphyrin) and with four carboxyl groups (coproporphyrin) but smaller amounts of porphyrins with seven six five and three carboxyl groups are also formed

## METABOLISM OF PORPHYRINS AND PORPHYRIN PRECURSORS IN THE INTACT ORGANISM

The bone marrow of an adult individual forms approximately 300 mg heme per day This amount is needed for hemoglobin formation to compensate for the physiologic decay of aged erythrocytes [13a] Additional heme is synthesized for the formation of other heme proteins but the lack of precise information regarding the rate of turnover of these compounds precludes exact estimation of the total daily heme production Undoubtedly it is considerably larger than the 300 mg required for hemoglobin synthesis in the bone marrow since heme containing compounds which are believed to be synthesized *in loco* are present in almost all aerobic cells [60] Relative to this rate of heme formation the excretion of porphyrins and of porphyrin precursors in urine and bile is very small If one visualizes the excreted compounds as the sum of all porphyrins and porphyrin precursors which escape during heme formation it becomes obvious that heme biosynthesis is proceeding with a remarkable degree of efficiency It is not known how much individual organs contribute to the

[130] that with ALA or PBG as substrates addition of mitochondria to a system consisting of hemolyzed human erythrocytes greatly enhances the formation of protoporphyrin III (9a) Conversion of coproporphyrinogen III to protoporphyrin III (9a) not only involves decarboxylation of the two propionic acid side chains but it requires formation of two vinyl groups It is therefore not surprising that in vitro biosynthesis of protoporphyrin III (9a) from ALA [131] PBG [108] uroporphyrinogen III [118] or coproporphyrinogen III [129] requires either aerobic conditions or the presence of some electron acceptor

Protoporphyrin III (9a) is used for the formation of heme It is not known whether iron is inserted at an intermediate stage of autoxidation of protoporphyrinogen or into protoporphyrin itself The latter seems more likely since it is well known that ferrous iron readily coordinates with porphyrins but not with porphyrinogens [113 132] In vitro heme may be formed nonenzymatically by simple incubation of protoporphyrin with ferrous iron at 37°C under nitrogen [132] On the other hand Krueger et al [133] and Goldberg [134] have presented evidence which suggests that in vivo incorporation of iron may be catalyzed by an enzyme system present in nucleated erythrocytes

## RECAPITULATION

The nearly complete elucidation of the pathways by which glycine and succinate are used for the biosynthesis of porphyrins and of heme provides an impressive example of the biochemical progress made during the last decade There can be little doubt that the few remaining regions of uncertainty and of ignorance will soon be explored and clarified This applies particularly to the enzymatic mechanism which catalyzes the condensation of 4 moles of PBG to form the asymmetric uroporphyrinogen III and also to the steps involved in the oxidative decarboxylation of coproporphyrinogen III to protoporphyrin III (9a)

Some aspects of the biosynthetic pathway which in the intact organism are believed to be of particular importance with regard to porphyrin metabolism are summarized below

1 While most of the individual biosynthetic steps proceed in the absence of oxygen two reactions require aerobic conditions (*a*) the formation of succinyl coenzyme A which is dependent on a functioning tricarboxylic acid cycle (*b*) the oxidative decarboxylation of coproporphyrinogen III to protoporphyrin III (9a)

2 These two reactions are catalyzed by enzyme systems which are bound to cell particles and which appear to be absent in mature nonnucleated erythrocytes On the other hand the steps from succinyl CoA to coproporphyrinogen III are catalyzed by soluble enzymes present both in nucleated cells and in mature mammalian erythrocytes

3 The following cofactors are essential pantothenate for the formation

PORPHYRINS

Normal human urine contains small amounts of porphyrins The predominant one is coproporphyrin which is excreted at a daily rate of 100 to 300 μg [148] the values being somewhat smaller in the female [149] Both types I and III isomers are present but the ratio is subject to considerable variation [151 152] In a variety of conditions such as hemolytic anemia liver disease and lead poisoning a slight to moderate increase in urinary coproporphyrin excretion is observed [148] and significant changes in isomer ratio have been reported [18] Coproporphyrin is also present in the bile where the daily excretion rate is estimated at 400 to 1 000 μg [148] Thus elevated values for urinary coproporphyrin excretion may result not only from increased porphyrin production but also from liver damage leading to diversion of excretion from the biliary to the urinary route [18] For example Hoffbauer and his coworkers [153] have found that in rats injected coproporphyrin III is entirely eliminated by the bile unless the bile ducts are occluded or the liver damaged by carbon tetrachloride

Other porphyrins present in normal human urine include uroporphyrin [148 154] and traces of porphyrins with seven six five and three carboxyl groups [151 155 156] Most of the uroporphyrin appears to be of type I isomer with only trace amounts of uroporphyrin III [154 157]

Protoporphyrin is normally absent from the urine [18 148] but is present in bile and feces [18 158] Even in Sedormid poisoning where very large amounts of protoporphyrin are excreted via the bile this porphyrin could not be demonstrated in the urine [34] As a general rule it can be said that ALA PBG and uroporphyrin are mainly excreted in the urine while coproporphyrin is preferentially and protoporphyrin exclusively eliminated via the bile

A large fraction of the porphyrins present in normal urine is excreted in the form of *precursors* which are converted to porphyrins by exposure to light and air or more efficiently by oxidation with dilute iodine [159–161] These precursors include probably porphyrinogens PBG and possibly ALA all of which can be converted nonenzymatically to porphyrins [100 144 162] It appears likely that the quantity and isomer type of porphyrins formed from precursors in the urine depends to some extent on external factors such as pH of the urine and type of oxidizing agent employed [122]

Except for circulating erythrocytes information regarding the concentration of free porphyrins in human tissues is scant Erythrocytes contain approximately 15 to 60 μg protoporphyrin and 1 to 2 μg coproporphyrin per 100 ml of cells [148 163] These values may be greatly increased in iron deficiency hemolytic anemia lead poisoning and other disorders of erythropoiesis [18 39 148 164]

'pool' of porphyrins and porphyrin precursors that are excreted but it appears likely that the bone marrow and the liver are major contributors

### δ-AMINOLEVULINIC ACID AND PORPHOBILINOGEN

In man approximately 2 mg ALA is excreted in the urine per day [19 87 136 137] No data are available for its excretion in bile but it is believed to be negligible [82 138] Significant elevation of ALA excretion in the urine is observed in lead poisoning [139] On the other hand Sedormid poisoning in rabbits does not appear to result in increased ALA excretion [37] although the concentration of PBG in the urine is high [36]

Injection or oral administration of labeled ALA to man and to animals results in the excretion of a large portion of the unaltered compound in the urine [82 138 140 141] Smaller fractions are converted to PBG, to protoporphyrin and to stercobilin the former being excreted in the urine the latter two in the bile [82, 138 140] All three compounds are believed to be formed outside the hematopoietic system, possibly in the liver [138, 140] In addition ALA 1 4 $C^{14}$ gives rise to labeled $CO_2$ in the breath [82] A small fraction of the injected ALA is used for heme biosynthesis in immature red blood cells [70 82 138 140] but in contrast to the observations in vitro incorporation of ALA into heme of circulating erythrocytes is several times less than that found with equivalent amounts of labeled glycine [82 138 140] This may in part be explained by the rapid renal excretion of the injected ALA either in unchanged form or after its conversion to PBG In addition penetration of ALA into immature red blood cells may be a rate-limiting factor [82 138 140]

In healthy individuals daily excretion of PBG in the urine is approximately 1 0 to 1 5 mg [19 87 136 137] At the low concentrations which exist in normal urine the conventional test for detection [142] is inadequate and gives negative results [143] Increased urinary excretion of PBG at times is observed in patients with hepatic malignant or infectious diseases [144] and is frequently present in lead poisoning [139 147] In animals poisoned with Sedormid [36] or related compounds [37 145] large amounts of PBG are excreted in the urine and are present in the liver but not in the bone marrow or circulating erythrocytes [36]

If injected parenterally PBG is rapidly excreted in the urine [146], fecal excretion or conversion of PBG to porphyrins is insignificant [141, 146] In animals injected PBG cannot be demonstrated in the liver [146] suggesting that it may not pass the liver cell membrane Similar findings have been reported for fowl erythrocytes in vitro [96] It is evident however that the impermeability of the cell membrane is only relative since in instances where endogenous PBG production is increased it does escape from the cells and gain access to the urine Thus in acute porphyria of man and in Sedormid porphyria of animals large amounts of PBG are derived from the liver and excreted in the urine [36 41 146]

PORPHYRINS

Normal human urine contains small amounts of porphyrins The predominant one is coproporphyrin which is excreted at a daily rate of 100 to 300 μg [148] the values being somewhat smaller in the female [149] Both types I and III isomers are present but the ratio is subject to considerable variation [151 152] In a variety of conditions such as hemolytic anemia liver disease and lead poisoning a slight to moderate increase in urinary coproporphyrin excretion is observed [148] and significant changes in isomer ratio have been reported [18] Coproporphyrin is also present in the bile where the daily excretion rate is estimated at 400 to 1 000 μg [148] Thus elevated values for urinary coproporphyrin excretion may result not only from increased porphyrin production but also from liver damage leading to diversion of excretion from the biliary to the urinary route [18] For example Hoffbauer and his coworkers [153] have found that in rats injected coproporphyrin III is entirely eliminated by the bile unless the bile ducts are occluded or the liver damaged by carbon tetrachloride

Other porphyrins present in normal human urine include uroporphyrin [148 154] and traces of porphyrins with seven six five and three carboxyl groups [151 155 156] Most of the uroporphyrin appears to be of type I isomer with only trace amounts of uroporphyrin III [154 157]

Protoporphyrin is normally absent from the urine [18 148] but is present in bile and feces [18 158] Even in Sedormid poisoning where very large amounts of protoporphyrin are excreted via the bile this porphyrin could not be demonstrated in the urine [34] As a general rule it can be said that ALA PBG and uroporphyrin are mainly excreted in the urine while coproporphyrin is preferentially and protoporphyrin exclusively eliminated via the bile

A large fraction of the porphyrins present in normal urine is excreted in the form of *precursors* which are converted to porphyrins by exposure to light and air or more efficiently by oxidation with dilute iodine [159–161] These precursors include probably porphyrinogen PBG and possibly ALA all of which can be converted nonenzymatically to porphyrins [100 144 162] It appears likely that the quantity and isomer type of porphyrins formed from precursors in the urine depends to some extent on external factors such as pH of the urine and type of oxidizing agent employed [122]

Except for circulating erythrocytes information regarding the concentration of free porphyrins in human tissues is scant Erythrocytes contain approximately 15 to 60 μg protoporphyrin and 1 to 2 μg coproporphyrin per 100 ml of cells [148 163] These values may be greatly increased in iron deficiency hemolytic anemia lead poisoning and other disorders of erythropoiesis [18 39 148 164]

# PHARMACOLOGIC ACTION OF PORPHYRINS AND OF PORPHYRIN PRECURSORS

## PHOTOSENSITIVITY

The photosensitizing activity of porphyrins is well known and has been the subject of many studies [22 165–169] This property is probably related to the intense fluorescence of porphyrins [22] since both are produced most effectively by long wave ultraviolet radiation of about 400 mμ wavelength corresponding to the peak absorption of porphyrins in the ultraviolet region of the spectrum [22 170–172] Uroporphyrin is believed to exhibit the strongest sensitizing effect followed by coproporphyrin, protoporphyrin has few if any photosensitizing properties [22 167 172 173] In the famous self-experiment of Meyer Betz [168] 200 mg hematoporphyrin was injected intravenously this resulted in marked erythema and edema of the exposed parts of the body Similar observations have recently been reported by Schwartz [174] The photodynamic effect in the skin may be mediated by release of histamine [175]

In spite of the recognized relation between porphyrins and photosensitivity [166] the pathogenesis of hydroa aestivale the vesicular eruption occurring on exposed parts of patients who suffer from photosensitive porphyria is not fully understood Blum and his coworker [170] failed to produce the characteristic vesicles by exposing areas of skin of a patient with erythropoietic porphyria to ultraviolet light although the wavelength of the emitted light corresponded to the absorption maximum of porphyrins Similar negative results were reported by Watson and by Gray [22 176] It appears likely that only continued and chronic exposure to sunlight results in formation of vesicles This is in line with the observation made in some of the patients with photosensitive porphyria that hydroa aestivale may not become manifest until midsummer but that the vesicular lesions then persist well into the fall [177]

Administration of ALA to human beings results in marked but transient hypersensitivity to sunlight [82 140] In rats injection of ALA followed by exposure to radiation from a carbon arc lamp produces marked photosensitivity [178] injection of PBG has no such effect This difference is believed to be related in part to the rate at which these compounds can enter epidermal cells [178] Fluorescence microscopic examination of skin obtained from animals injected with ALA suggest that ultraviolet radiation may enhance the intracellular conversion of ALA to uroporphyrin [178] This is reminiscent of the photocatalytic autoxidation of porphyrinogens to porphyrins in vitro a process which is sensitized by the product porphyrin [105] In vivo de Mello [179 180] observed that in rabbits poisoned with lead acetate or injected with the photosensitizing dye rose bengal ultraviolet irradiation results in a

marked increase in urinary coproporphyrin excretion In analogy with the observations in vitro it is tempting to attribute this extra excretion of pigment to photocatalytic conversion of precursors to porphyrins [181] It is not clear however why the majority of patients with acute porphyria are free from photosensitivity, although large amounts of porphyrin precursors are present in the liver [41] and are excreted in the urine

The above findings may be summarized as follows Increased concentration of preformed porphyrins in the body undoubtedly predispo es to cutaneous photosensitivity Light of near ultraviolet wavelength is particularly effective Vesicular eruptions of the skin are produced only after prolonged and chronic exposure to sunlight Under experimental conditions irradiation with ultraviolet light appears to promote conversion of porphyrin precursors to porphyrins resulting in photosensitivity and increased porphyrin excretion in the urine These observations suggest that photosensitivity is a *relative* phenomenon and may depend on such factors as light source length and intensity of exposure types of porphyrins and of precursors present and their relative concentration in the skin and in the extracellular fluid

### OTHER PHARMACOLOGIC EFFECTS

In addition to their photosensitizing properties porphyrins are said to produce a variety of toxic effects including vascular and intestinal spasm and neuropathologic changes [*47 182 183*] Reinvestigations of the pharmacologic action of naturally occurring porphyrins however have failed to produce convincing evidence which would suggest demonstrable effects of these compounds on smooth muscles or on the nervous system [*178 184–186*] Further neither injection of hematoporphyrin [*168*] nor excessive production of endogenous uroporphyrin as in congenital erythropoietic porphyria gives rise to intestinal or neurologic symptoms Nor are such symptoms produced by injection of PBG or ALA in man or in animals [*82 140 178 184 185 187*] In vitro these compounds were found to be without effect on smooth muscle of rat intestine [*178 184*]

In view of the e negative findings it appears doubtful whether the intestinal vascular and neurologic manifestations of acute porphyria can be ascribed to direct toxic effects of porphyrins or of porphyrin precur ors [*121 178 181*]

## CONGENITAL ERYTHROPOIETIC (PHOTOSENSITIVE) PORPHYRIA

### INCIDENCE AND CLINICAL MANIFESTATIONS

Erythropoietic porphyria is a rare congenital di ease occurring much less frequently than other forms of porphyria The prominence of photo-

sensitivity and of massive porphyrinuria has often caused it to be confused with the hepatic types of cutaneous porphyria [*18*], although the pathogenesis of the latter disorders is fundamentally different [*41*]. In the earlier literature [*13, 188*] many of the reported cases clearly had features of the hepatic forms while in other instances the data were insufficient to permit distinction [*15*]. A reexamination of the cases of so-called congenital photosensitive porphyria reported up to 1954 permitted the diagnosis of erythropoietic porphyria beyond reasonable doubt in a total of 34 cases [*15*]. An additional 7 cases have since come to the author's attention. These 41 cases and some of their essential features are summarized in Table 30-1. Twenty four of the patients were female and 17 male. In the earlier literature it was generally held that congenital porphyria was more common in males, but this was undoubtedly caused by confusion with porphyria cutanea tarda, which is more frequent in males [*38–40*]. As seen in Table 30-1, the disease has a wide racial distribution and has been observed in children of Japanese, Bantu and Sudanese extraction [*195, 199, 202, 219, 225, 226*].

The first sign suggesting the presence of erythropoietic porphyria is usually the excretion of red urine containing much uroporphyrin but no PBG. This may be noted at birth or only during the first years of life. Although porphyrinuria is probably present at all times, the amounts of porphyrins excreted and hence the red color of the urine may show considerable daily or seasonal fluctuation [*8, 41, 203*]. In some instances the color of the urine was light except during periods of active photodermatitis occurring during the summer months [*8, 41, 203*]. In others large and relatively constant amounts of uroporphyrin were excreted over long periods of time [*13*].

Photosensitivity is frequently absent in the neonatal period, but it may become apparent during the first years of life as exposure to sunlight increases. A vesicular or bullous eruption appears on the face, the back of the hands and other exposed parts of the body. This is commonly referred to as hydroa aestivale, the adjective indicating the seasonal recurrence of the lesions. The vesicles contain a serous fluid which may exhibit red fluorescence, heal slowly and leave depressed pigmented scars. Infected bullae often ulcerate, causing marked scarring and deformity involving particularly the tips of the fingers, the ears, nose and eyelids. After years of repeated attacks severe mutilation may ensue, with contractions of the face and loss of parts of digits and ears [*13*]. In other cases the cutaneous manifestations may be relatively mild, resulting in little if any scarring. In several patients a definite decrease in photosensitivity and a reduction in urinary porphyrin excretion followed splenectomy [*41, 203, 207*]. In one of these children [*203*] removal of the spleen resulted in virtual loss of photosensitivity, but porphyrin-containing vesicles were observed on those parts of the hands which became infested with scabies [*177*].

Hypertrichosis is a frequent finding in these patients [13 22] Fine blond downy hair resembling lanugo may cover the face and extremities [13 22 190 204 214] Deciduous and permanent teeth may show a red or brownish discoloration (erythrodontia) [13 202] but in some cases this has been rather inconspicuous [18] Under ultraviolet light however the teeth always exhibit marked red fluorescence Deposition of porphyrin in the developing teeth and in bones is believed to be due to its physical affinity for calcium phosphate [18] The presence of porphyrin in the deciduous teeth may indicate that the metabolic disorder was already present during fetal life [191]

Splenomegaly is an almost constant feature of the disease (Table 30 1) It may be absent in the neonatal period and be detected only as the patient grows older [15] In one patient the spleen was enlarged in early childhood but could no longer be palpated 10 years later [192] In three patients splenomegaly was absent this was correlated with normal erythrocyte survival and lack of hemolytic anemia [217 225] Hypertension and abdominal or neurologic symptoms so frequently observed in other forms of porphyria are not found in erythropoietic porphyria

## HEMATOLOGIC FINDINGS

In the majority of the reported cases increased hemolytic activity was present as indicated by the following findings normochromic anemia associated with elevated reticulocyte levels and circulating normoblasts normoblastic hyperplasia of the bone marrow increased excretion of fecal urobilinogen [15] Rarely the anemia was severe leading to early death [195] or requiring multiple transfusions [227] but in most patients the reduction in hemoglobin concentration was only slight and the hemolysis was largely compensated by increased red cell production It was stated that the famous case Petry [202] at the time of his death suffered from pernicious anemia in addition to congenital porphyria but Aldrich et al [203] raised justified doubt as to the correctness of this interpretation of the autopsy findings and suggested that Petry may have had an unrecognized hemolytic anemia with splenomegaly [15] In another patient originally reported by Ashby [196] and later by Garrod [197] outspoken hemolytic anemia was repeatedly demonstrated [197] At the time of her death at age 27 she was found to have a coarse-nodular cirrhosis of the liver and the bone marrow showed extensive myeloid hyperplasia and no significant erythroid haemopoiesis [197] The available information is inadequate to permit speculation as to the possible factors which could have led to this marrow failure [15]

Direct estimation of erythrocyte life span has been performed in seven patients [210 212 214 217 228 229 230] five of whom had hematologic findings suggestive of hemolytic anemia A decreased survival of autologous erythrocytes was demonstrated by the glycine-$N^{15}$ method in the two patients studied by Grinstein et al [228] and Watson et al [212]

TABLE 30 1 CASES OF CONGENITAL ERYTHROPOIETIC PORPHYRIA REPORTED IN THE LITERATURE UP TO 1958

| Series or author | Year | Essential facts of patient | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|
| | | Sex | Racial background | Age at first symptoms | Anemia | Splenomegaly | Erythrodontia | |
| And s [8] | 1898 | M | English | 4 | 0 | 0 | 0 | Two brothers ... t r bel ... lt haved ed with i l ray ... t ms |
| A d [9] | 1898 | M | English | 3 | 0 | 0 | 0 | |
| R d h [189] | 1911 | F | Italian | 14( ) | Hypochr | 0 | 0 | Time of appearance of first symptoms not clearly stated |
| C pp lli [190] | 1914 | M | Italian | 3 | + type not stated | + | 0 | |
| M k y [191 192] | 1922 | M | English | Birth | Hemolytic | + | + | Spl ... to y ... ed 1932 ... [193] |
| M k y [191] | 1922 | M | English | 2 | Hemolytic | + | + | |
| G y [192 194] | 1924 | F | English | 5 | Hemolytic | + | + | |
| S t [195] | 1926 | M | Jap | Birth | Hypoch | + | + | |
| Ashby [192 196] | 1926 | F | English | Birth | Hemolytic | + | + | Se ... l ... ged ... with sim l ray ... t ms. Died 1949 ... f ll w ... ry [197] |
| Schmidt [198] | 1926 | F | German | 2 | Hemolytic | + | 0 | Spl ect my ... t n t pe f r ... |
| K t g w [199] | 1927 | F | Japanese | Birth | Hemolytic | + | 0 | S t of the f ll w g ca |
| Mat k [200] | 1928 | M | Japanese | 16( ) | Hemolytic | + | 0 | Br th ... t m of ... symptoms n t ... rly t ted |
| M t ok [200] | 1928 | F | Japanese | 3 | Hemolytic | + | + | |
| M [201] | 1929 | M | Italian | Birth | + type not stated | + | + | |
| B t [202] | 1929 | M | German | Birth | + | + | + | C ... l try ... is beli ... l to be ... b t m y l ... teen hem lytic [203] |
| M rl [204] | 1931 | M | Italian | 5(?) | Hemolytic | + | + | St d ... g in in 1948 [205] |
| T g [171 206] | 1933 | M | White | 1 | + type not stated | + | 0 | |
| De M l [207] | 1934 | F | A g t | 5 | H lyt | + | 0 | Spl ectomy w th p t l ... f ... to- se t ity |
| He d [208] | 1938 | F | Spanish | 1 | 0 | 0 | 0 | Two ... t ... |
| He n d [208] | 1938 | F | Spanish | 1 | 0 | 0 | 0 | |
| H d [208] | 1938 | F | Spanish | Early ... lf | 0 | 0 | 0 | S bl g ... ly ... ti ... ed u ... hy pe t ... |
| Her do [209] | 1938 | F | Spanish | Early infancy | 0 | 0 | 0 | |
| H d [208] | 1938 | M | Spanish | Early ... lf | 0 | 0 | 0 | |

TABLE 30-1 CASES OF CO CENITAL FRYTHROPOIETIC PORPHYRIA REPORTED IN THE LITERATURE UP TO 1958 (*Continu d*)

| S w th | Y | E t al f t f pat t | | | | | | R m k |
|---|---|---|---|---|---|---|---|---|
| | | S | R al ba kg d | Ag at f ympt m b | A mia | Spl o m galy | Eryth o d tia | |
| P h y [209] | 1938 | F | Wh t | B th | H m lyt | + | + | L t tud ed by Lo d West St m o R t t nbe g [ ICI |
| D f r [211] | 1938 | F | Wh t | 3 | + typ t t ted | + | + | Spl tom d 1957 th m n f h m lyt m [212] |
| D ky [213] | 1947 | F | Wh t | B th | 1 sed h m ly | + | + | Spl t my 1953 214] |
| M y [215 216] | 1948 | M | F h | F t 2 y f1f | – | – | + } | N mal g I f ch m t d yth yt [21 ] |
| M y [215 216] | 1 48 | F | F h | F t 2 y f1f | – | – | + } | |
| M y [215 216] | 1948 | M | F h | F t 2 y f1f | H m lyt | + | + | B th f abo t sa |
| C l tt [213] | 1948 | M | It l | 3 4 | + moch | 0 | 0 | 11 t lthy bl g |
| Fi dl y [219 220] | 19 0 | F | B t | 11 (m ) | H lyt | + | + | Par t f t |
| Ald h [ 03] | 1951 | F | No w g | 2 | H m lyt | + | + | Spl tomy th m f ph tose m t ty |
| Z l r [221 222] | 1951 | F | Wh t | Du g h t yea f1f | H lyt | + | + | Spl t my th p t l m se f ph to- e t ty |
| P [223] | 1953 | F | It l | B th | H m lyt | + | + | S ph tod matt th n g |
| S hm d [15 4 ] | 1954 | F | F gl h | 3 | H m lyt | + | + | Spl ect my w th p t l ss f ph to- se t vity |
| W mo [ 24] | 1954 | F | I lish | 1 | H m lyt | + | + } | Ol t a d yo g t f f ur t l g |
| W m [224] | 1954 | F | l ol h | B t) | H m lyt | + | + } | |
| T w se d C les [220 225] | 195 | M | S nd | 9 (m ) | – | – | + | N m blast c hype pla f bo m rr w |
| T w se d-C les [220 25] | 1957 | F | S d n se | 7 ( ) | H m yt | + | + | S t f bo case |
| B ter [226] | 19 8 | F | B t | B th | H m lyt | + | + | Spl ectomy with p rtl l m ss f ph to- se t vity |
| St h [227] | 19 8 | M | G m n | B th | H m lyt | + | + | Spl ectomy f se v h m lyt c mi |

K: + = pr sent – = bsent 0 = t t m t
N te See al o Add d m t ti e d f th t pt

TABLE 30.1 CASES OF CONGENITAL ERYTHROPOIETIC PORPHYRIA REPORTED IN THE LITERATURE UP TO 1958

| Senior author | Year | Essential features of patient | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|
| | | Sex | Racial background | Age at onset of symptoms, yr | Anemia | Spleno-megaly | Erythro-dontia | |
| And rs [9] | 1898 | M | English | 4 | 0 | 0 | 0 | Two brothers … both … with … symptoms |
| A d [9] | 1898 | M | English | 3 | 0 | 0 | 0 | |
| Rad h [189] | 1911 | F | Ital | 14( ) | Hyperchromic | 0 | 0 | Time of appearance of first symptom not clearly stated |
| C pp ll [190] | 1914 | M | Italian | 3 | + type not stated | + | 0 | |
| M key [191, 192] | 19 | M | English | Birth | Hemolytic | + | + | … died 193 in … [191] |
| M k y [191] | 19 | M | English | | Hemolytic | + | + | |
| G y [192, 194] | 1924 | F | English | 5 | Hemolytic | + | + | |
| S to [195] | 19 6 | M | Japanese | Birth | Hypochromic | + | + | |
| A hby [192, 196] | 19 6 | F | English | Birth | Hemolytic | + | + | … died with … symptom; Died 1949 … ry [197] |
| S hm dt [198] | 19 6 | F | German | 2 | Hemolytic | + | 0 | Splenectomy … but not performed |
| Kitag [199] | 1927 | F | Japanese | Birth | Hemolytic | + | 0 | Sister of following case |
| M ts ka [200] | 19 8 | M | Japanese | 16( ) | Hemolytic | + | 0 | Brother of preceding … first symptoms … stated |
| M t u ka [200] | 19 8 | F | Japanese | 3 | Hemolytic | + | + | |
| Mar [201] | 19 9 | M | Italian | Birth | + type not stated | + | + | |
| B rst [202] | 19 9 | M | German | Birth | + | + | + | Case … reported … but … been hemolytic [203] |
| M n [204] | 1931 | M | Italian | 5( ) | Hemolytic | + | + | Studied again in 1948 [205] |
| T ss g [171, 206] | 1933 | M | White | 1 | + type not stated | + | 0 | |
| De Ma ll [207] | 1934 | F | Argentine | 5 | Hemolytic | + | 0 | Splenectomy with partial remission of photosensitivity |
| H d [208] | 1938 | F | Spanish | 1 | 0 | 0 | 0 | Two … ; … urine … porphyrins |
| H nd [208] | 1938 | F | Spanish | 1 | 0 | 0 | 0 | |
| H rn d [208] | 1938 | F | Spanish | Early life | 0 | 0 | 0 | |
| H d [208] | 1938 | F | Spanish | Early life | 0 | 0 | 0 | |
| H d [208] | 1936 | M | Spanish | Early life | 0 | 0 | 0 | |

by Canivet et al [217] exhibited marked hemolytic anemia [215 216] Furthermore between the two Sudanese siblings studied by Townsend Coles and Barnes [22?] one had marked hemolytic anemia while in the other hemoglobin concentration erythrocyte count and reticulocyte count were normal although the bone marrow showed normoblastic hyperplasia

The mechanism responsible for the hemolytic process is not understood The absence of a positive Coombs test and the finding of decreased survival of porphyric cells in a normal recipient [214] may be suggestive of an intracorpuscular defect On the other hand Hausmann s observation [232] that hematoporphyrin sensitizes normal red cells to hemolysis by light in vitro gave rise to the speculation that the presence of porphyrins in the circulating plasma may exert a hemolytic effect in vivo [229] An alternative explanation is suggested by the observations made in the fluorescence microscope that only a fraction of the normoblasts and of the immature erythrocytes contains increased amounts of porphyrins [15] If the presence of porphyrins predisposes erythrocytes to hemolysis it is conceivable that porphyrin laden cells may have a shortened life span whereas cells which lack excessive amounts of porphyrin may survive normally The presence of very high porphyrin concentrations in porphyric spleens [41 214] is compatible with this hypothesis It is possible that the life span of porphyrin containing cells may be further shortened by exposure of the patients to sunlight It should be noted however that none of these explanations is based on concrete experimental evidence

Studies of unstained bone marrow preparations in the fluorescence microscope show intense porphyrin fluorescence in nucleated red cells and to a lesser degree in reticulocytes [15 41 212] (Fig 30 11) In normoblasts most of the porphyrin appears to be present either inside or at the surface of the cell nucleus (Fig 30 11) [15] A fraction of the normoblasts in all developmental stages and some of the reticulocytes in the peripheral blood fail to exhibit any red fluorescence [15] There appears to be a clear separation between fluorescing and nonfluorescing cells Furthermore fluorescing normoblasts exhibit a morphological abnormality in nuclear structure consisting of single or at times multiple nuclear inclusions (Fig 30 12) which stain dark with benzidine (Fig 30-13) and which show strong absorption in the 400 mμ band [15] This nuclear abnormality is not present in normoblasts which lack porphyrin fluorescence It is interesting to note that similar findings have been reported in congenital porphyria of cattle [212] These observations suggest the possibility that two different lines of erythropoietic cells may be present and that only one exhibits the defect in porphyrin metabolism [15]

In patients in whom increased hemolysis was demonstrable the excre

In the former patient splenectomy resulted in remarkable improvement in hemoglobin concentration porphyrin excretion, and photosensitivity [203] in the latter patient, the time which has elapsed since the operation is too short to permit adequate evaluation In the case reported by London et al [210] the average life span of the erythrocytes appeared to be normal but the reticulocyte count was elevated and the marrow showed erythroid hyperplasia In a study reported by Rosenthal et al [214] erythrocytes of a patient with erythropoietic porphyria were infused into a recipient with ulcerative colitis, and the disappearance of the donor s cells from the recipient s circulation was followed by differential agglutination Although the analysis of the results was complicated by the fact that the recipient was bleeding the findings suggested a more rapid rate of removal of the porphyric cells as compared to cells from a normal donor Splenectomy in this patient resulted in only temporary improvement although 4 years postoperatively survival of chromated erythrocytes was almost normal [231] On the other hand in two siblings studied by Canivet et al [217] red cell survival as estimated by the $Cr^{51}$ method appeared to be normal and there was no evidence of hemolytic anemia as judged by normal hemoglobin concentration reticulocyte counts fecal urobilinogen excretion and absence of splenomegaly and of erythroid hyperplasia of the bone marrow

These seemingly contradictory results may find a possible explanation in the observation that a hemolytic component may be present during one phase of the disease but absent at other times Thus in a patient in whom over the course of 25 years hemolytic anemia had repeatedly been demonstrated [191 192] Gray et al [229 230] found that approximately half the erythrocytes appeared to have a mean life span of less than 20 days while other red cell fractions survived for periods of 40 to 70 days and for 110 to 120 days respectively At the time of this study the patient was believed to have a hemolytic crisis as suggested by the presence of anemia reticulocytosis normoblasts in the peripheral blood and increased excretion of fecal urobilinogen At a later date when the reticulocyte count had returned to normal a second study of erythrocyte survival suggested that at least 80 per cent of the cells appeared to have a normal life span while only a minor fraction appeared to be removed between the thirtieth and the eightieth day All these values were calculated on the basis of data obtained by following the $N^{15}$ content of circulating hemoglobin heme after administration of $N^{15}$ labeled glycine (Fig 30 2) [229 230] The limitations of this method for determining the apparent life span of circulating erythrocytes must be kept in mind

The above findings suggest that increased hemolysis though present in most of the cases may be intermittent in nature and at times may be so slight as to be difficult to detect It is interesting that the porphyric brother of the two patients with normal erythrocyte survival reported

by Canivet et al [217] exhibited marked hemolytic anemia [215 216] Furthermore between the two Sudanese siblings studied by Townsend Coles and Barnes [220] one had marked hemolytic anemia while in the other hemoglobin concentration erythrocyte count and reticulocyte count were normal although the bone marrow showed normoblastic hyperplasia

The mechanism responsible for the hemolytic process is not understood The absence of a positive Coombs test and the finding of decreased survival of porphyric cells in a normal recipient [214] may be suggestive of an intracorpuscular defect On the other hand Hausmann s observation [232] that hematoporphyrin sensitizes normal red cells to hemolysis by light in vitro gave rise to the speculation that the presence of porphyrins in the circulating plasma may exert a hemolytic effect in vivo [229] An alternative explanation is suggested by the observations made in the fluorescence microscope that only a fraction of the normoblasts and of the immature erythrocytes contains increased amounts of porphyrins [15] If the presence of porphyrins predisposes erythrocytes to hemolysis it is conceivable that porphyrin laden cells may have a shortened life span whereas cells which lack excessive amounts of porphyrin may survive normally The presence of very high porphyrin concentrations in porphyric pleens [41 214] is compatible with this hypothesis It is possible that the life span of porphyrin containing cells may be further shortened by exposure of the patients to sunlight It should be noted however, that none of these explanations is based on concrete experimental evidence

Studies of unstained bone marrow preparations in the fluorescence microscope show intense porphyrin fluorescence in nucleated red cells and to a lesser degree in reticulocytes [15 41 212] (Fig 30 11) In normoblasts most of the porphyrin appears to be present either inside or at the surface of the cell nucleus (Fig 30 11) [15] A fraction of the normoblasts in all developmental stages and some of the reticulocytes in the peripheral blood fail to exhibit any red fluorescence [15] There appears to be a clear separation between fluorescing and nonfluorescing cells Furthermore fluorescing normoblasts exhibit a morphological abnormality in nuclear structure consisting of single or at times multiple nuclear inclusions (Fig 30 12) which stain dark with benzidine (Fig 30-13) and which show strong absorption in the 400 mμ band [15] This nuclear abnormality is not present in normoblasts which lack porphyrin fluorescence It is interesting to note that similar findings have been reported in congenital porphyria of cattle [212] These observations suggest the possibility that two different lines of erythropoietic cells may be present and that only one exhibits the defect in porphyrin metabolism [15]

In patients in whom increased hemolysis was demonstrable the excre

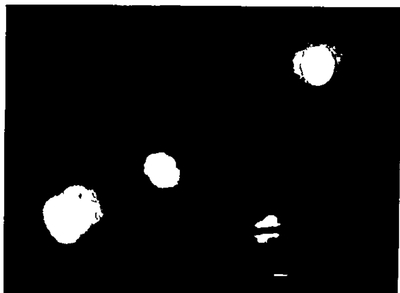


Fig 30-11 Unstained bone marrow smear of a patient with erythropoietic porphyria photographed in the fluorescence microscope (× 800) Red fluorescence (reproduced in white) is particularly intense in the normoblastic nuclei The polychromatophilic erythrocyte in the left lower corner exhibits a lesser degree of porphyrin fluorescence (*By permission of I Schmid et al* [41])



Fig 30-12 Bone marrow smear from a patient with erythropoietic porphyria stained with Wright s stain The nucleus of the large normoblast shows a central inclusion (*By permission of I Schmid et al Acta haemat* 10 153 1953)

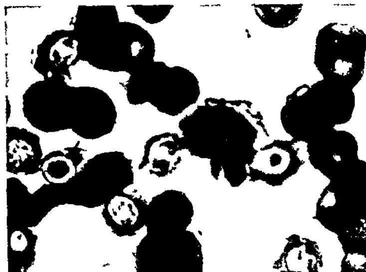


Fig 30-13 Bone marrow smear from a patient with erythropoietic porphyria stained with benzidine Abnormal normoblasts contain dark-staining nuclear inclusions Nuclei of normal immature normoblasts appear granular (left border) those of normal mature normoblasts white (left lower corner) Hemoglobin-containing erythrocytes stain dark (*By permission of R Schmid et al [15]*)

tion of fecal urobilinogen was elevated [*15 41 203 214 229*] More important was the observation that a major fraction of the excreted bile pigments was not derived from breakdown of hemoglobin of mature circulating erythrocytes [*65 210 228 230*] While in normal subjects 80 to 90 per cent of the fecal urobilinogen originates from destruction of red cells which have reached the end of their physiologic life span [*65 230 233*] (Figs 30 2 30 14) this does not apply to erythropoietic porphyria [*65 210 230*] At least 31 per cent and in some patients as much as 80 per cent of the excreted urobilinogen is derived from sources other than circulating hemoglobin (Fig 30 15) Most of this urobilinogen fraction is excreted during the first 20 days after the administration of labeled glycine (Fig 30-15) That it is not derived from destruction of circulating erythrocytes is demonstrated by the fact that there is no concomitant fall in labeled hemoglobin heme

The source of this early labeled urobilinogen fraction is obscure It appears unlikely that nonhemoglobin heme such as myoglobin and other heme proteins contribute significantly to its formation [*210 230 234*] It has been suggested that it may be derived from developing red cells which are destroyed in the marrow or shortly after their release into the circulation or alternatively that heme which is formed in excess of globin may be discarded and converted to bile pigments [*234 235*] As a third possibility it may be suggested that the increase in the

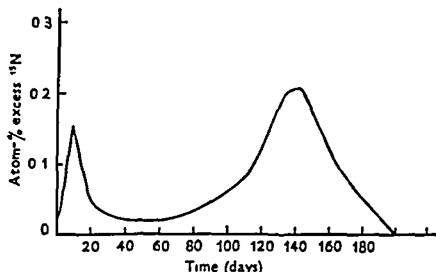



Fig. 30-14 Concentration of $N^{15}$ in fecal stercobilin of a normal subject given 1 gm glycine-$N^{15}$ (31.65 atom per cent excess $N^{15}$) during the first 4 days of the experiment. (*By permission of C. H. Gray [30].*)

Fig. 30-15 Concentration of $N^{15}$ in fecal stercobilin of a patient with erythropoietic porphyria given 1.2 gm glycine-$N^{15}$ (31.65 atom per cent excess $N^{15}$) during the first 4 days of the experiment. (*By permission of C. H. Gray [30].*)

early labeled urobilinogen fraction may be related to the presence of two types of erythroid cells [15] as demonstrated in the fluorescence microscope If it could be established that porphyrin containing erythrocytes have a much shorter life span than those which lack an increased pigment concentration the observed pattern of bile pigment excretion would find a simple explanation None of these hypotheses however is based on concrete experimental evidence

## CHEMICAL FINDINGS

The color of the urine of patients with erythropoietic porphyria may vary from a faint pink to a Burgundy red to a dark reddish brown depending upon the concentration of porphyrins The amounts of uroporphyrin excreted are always increased and daily excretion of as much as 500 mg has been reported [13] In addition the urine contains large amounts of coproporphyrin but the concentration is usually less than that of uroporphyrin [41, 203 236] Smaller amounts of porphyrins with seven six five and three carboxyl groups have also been demonstrated [217 227 236] Most of the urinary porphyrins are of the type I isomer [39 53] but small fractions of uroporphyrin III have been identified [236 237] For coproporphyrin the type I to type III isomer ratio may be as high as 99 1 [203] although small amounts of coproporphyrin III have been isolated in crystalline form [203]

PBG as identified by the conventional semiquantitative method [142] is consistently absent from the urine Using more refined techniques the daily excretion of PBG and of ALA in the urine is said to be within normal limits [227] The feces regularly contain large amounts of coproporphyrin [13 197 203 212 220 229] while the amount of uroporphyrin is much smaller [197 203 212] Fecal excretion of protoporphyrin III (9a) though variable is usually not elevated Most of the coproporphyrin is of type I isomer although small amounts of coproporphyrin III have been identified [197 203]

Variable concentrations of uroporphyrin I and coproporphyrin I are regularly demonstrable in the plasma [41 203 212 214] Circulating erythrocytes contain high concentrations of uroporphyrin I [41 203 212 214 227] and somewhat lower concentrations of coproporphyrin I while for protoporphyrin III (9a) the values are usually not higher than those found in other hemolytic conditions [238] Typical values in a patient studied before and after splenectomy are given in Table 30-2 Highest concentrations of these porphyrins are present in the bone marrow (Table 30-2) where the ratio of uroporphyrin I to coproporphyrin I is about the same as in the peripheral blood [41 212 227] Most of the bone marrow porphyrins are present in developing erythroid cells (Fig 30-11) [15 207] Uroporphyrin I in crystalline form has been isolated from bone marrow obtained by needle aspiration [41]

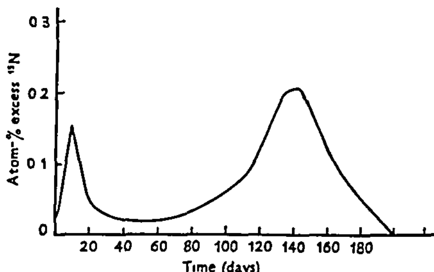



Fig. 30-14 Concentration of $N^{15}$ in fecal stercobilin of a normal subject given 1 gm glycine-$N^{15}$ (31.65 atom per cent excess $N^{15}$) during the first 4 days of the experiment (*By permission of C. H. Gray [30].*)

Atom-% excess ¹⁵N
1.0
0.75
0.50
0.25
0
20 40 60 80 100 120 140 160
Time (days)

Fig. 30-15 Concentration of $N^{15}$ in fecal stercobilin of a patient with erythropoietic porphyria given 12 gm glycine-$N^{15}$ (31.65 atom per cent excess $N^{15}$) during the first 4 days of the experiment (*By permission of C. H. Gray [30].*)

system which converts PBG to uroporphyrinogen III [*114* *124* *125*] Preheating of the system to 60 C however results in loss of the ability to form the type III isomer PBG being converted *only* to uroporphyrinogen I [*124* *125*]

In erythrocytes of patients with erythropoietic porphyria Rimington and Booij [*130*] and MacRae [*239*] demonstrated that ALA and PBG are converted to porphyrinogens of *both* type I and type III isomers Thus red cells in erythropoietic porphyria resemble *normal* human erythrocytes in which the ability to form porphyrins of type III isomer has been partly destroyed It is obvious that in porphyric erythrocytes the metabolic defect is only a partial one since complete inability to synthesize porphyrins of type III isomer would preclude the formation of hemoglobin a situation which would be incompatible with life Information as to the relative amounts of the two porphyrin isomers formed in porphyric cells is not yet available

It might be expected that in maturing porphyric red cells the highest rate of synthesis of uroporphyrinogen I would coincide with the phase of most active hemoglobin formation As Thorell [*240*] has shown this occurs in the later stages of the developing normoblast Part of the uroporphyrinogen I synthesized in these cells is oxidized to uroporphyrin I while another fraction is decarboxylated to porphyrinogens with less than eight carboxyl groups mainly to coproporphyrinogen I which in turn is converted to coproporphyrin I (Fig 30 9) The factors which determine the relative amounts of the e type I porphyrins formed in porphyric red cells are not known but it would appear that the amount of coproporphyrin I formed may depend in part on the rate at which uroporphyrinogen I is oxidized to uroporphyrin I Since this process is photocatalytic [*105*] and is sensitized by the product uroporphyrin it may provide a possible explanation for the observation that some of the patients with erythropoietic porphyria are found to excrete more uroporphyrin during the summertime when they are exposed to sunlight [*8* *41* *203*]

The mechanism by which porphyrins are released from the cells is not understood A simple calculation based on the concentration of porphyrins in the circulating red cells indicates that release of porphyrins from hemolyzed erythrocytes could not account for more than a relatively small fraction of the excreted pigments [*41*] The high porphyrin content of bone marrow suggests that a major fraction of the porphyrins present in plasma and excreted in urine and bile may be derived from developing red cells This is supported by the observation that the most intense porphyrin fluorescence is found in immature red cells which still have their nuclei [*15*] (Fig 30-11) The erythroid hyperplasia of the bone marrow resulting from the increased rate of hemolysis further augments the formation of type I porphyrins [*39* *41* *212* *227*] The beneficial

Large amounts of uroporphyrin I are also present in the spleen [41 202 203, 212 214 227] On fluorescence microscopic examination fine dustlike granules of porphyrin are visible in the red pulp but not in the Malpighian corpuscles [41, 202] In contrast to the marrow, splenic cells

TABLE 30-2 PORPHYRIN CONCENTRATIONS IN ERYTHROCYTES BONE MARROW AND URINE OF A 6-YEAR-OLD GIRL WITH ERYTHROPOIETIC PORPHYRIA

| *Date* | *Erythrocytes µg/100 ml* | | | *Bone marrow µg/100 ml* | | | *Urine µg/24 hr* | |
|---|---|---|---|---|---|---|---|---|
| | *URO* | *COPRO* | *PROTO* | *URO* | *COPRO* | *PROTO* | *URO* | *COPRO* |
| 11/3/50 | 110 | 56 | [illegible] | 2 635 | 302 | 121 | 8 450 | 2 780 |
| 4/2/51 | 377 | 91 | 41 | 2 432 | 614 | 190 | 21 500 | 3 450 |
| 6/21/51 | 410 | 150 | 105 | | | | 52 300 | 2 800 |
| 6/21/51 | Splenectomy spleen contained 3 400 µg URO/100 gm | | | | | | | |
| 7/3/51 | | | | | | | 1 200 | 400 |
| 10/6/51 | 192 | 20 | 60 | 170 | 81 | 281 | 6 900 | 2 310 |

NOTE URO COPRO PROTO = uroporphyrin coproporphyrin protoporphyrin respectively
SOURCE R Schmid et al [41]

do not exhibit nuclear fluorescence [41] In the liver red fluorescence is minimal but considerable amounts of uroporphyrin I are demonstrable on extraction [41 202 212 214]

## NATURE OF THE METABOLIC DEFECT

*The findings discussed in the preceding sections indicate that in erythropoietic porphyria* the primary defect probably involves overproduction of porphyrins of type I isomer for which the body has no use The defect appears to be confined to the hematopoietic system [15 41, 128 230] and may involve only part of the erythroid cells [15 202] Thus Fischer s original concept [53] that porphyrins of type I isomer are synthesized along with and parallel to hemoglobin formation appears to be essentially correct

As seen in Figs 30-8 30-9 the most likely site for the metabolic defect is in the enzymatic mechanism which converts PBG to uroporphyrinogen It may be recalled that PBG deaminase converts PBG only to uroporphyrinogen I whereas formation of uroporphyrinogen III requires the *combined* action of PBG deaminase and uroporphyrinogen isomerase It would appear that either overactivity of PBG deaminase *or* a defect in uroporphyrinogen isomerase would account for the increased formation of uroporphyrinogen I at the expense of the type III isomer [113 117] It has been noted that the latter enzyme is relatively heat labile [113, 114 117] Human red cell hemolysates contain the complete enzymatic

patients are homozygous for the trait The sexes are equally affected The lack of increased porphyrin excretion in parents and in normal siblings of patients [217 225] suggests that individuals who are heterozygous for the trait do not exhibit the metabolic abnormality Thus the mode of inheritance of erythropoietic porphyria appears to be autosomal recessive

CONGENITAL PORPHYRIA IN OTHER MAMMALS

**Cattle** Congenital porphyria has been observed in cattle in South Africa Denmark England and the United States [212 244–247] In all instances the disease was inherited as a simple Mendelian recessive characteristic Heterozygous animals are clinically and biochemically normal [212 246 247] Porphyric calves were obtained by breeding animals which were known to be heterozygous for the trait [212 248]

Afflicted animals exhibit photosensitivity of those areas of the skin which are not pigmented or covered with dark hair [212 248] The first case of bovine porphyria recognized in Denmark was discovered because the cow was not getting on at pasture but did well in the stable When the animals are slaughtered bones and teeth are found to be dark and to contain a large amount of porphyrin [212 244 246 248] The bovine form of congenital porphyria appears to have distinct similarities to the human disease Anemia reticulocytosis and splenomegaly may be present [212] but splenectomy performed in one porphyric cow failed to result in significant improvement [212] The spleen contained much porphyrin mostly uroporphyrin I the porphyrin concentration in the liver was insignificant [212 247] As in the human disease highest porphyrin concentrations were found in the bone marrow [212] and on fluorescence microscopic examination unstained bone marrow preparations exhibited intense porphyrin fluorescence which was most prominent in normoblastic nuclei On staining these cells exhibited abnormalities of nuclear structure similar to those described in human porphyria (Figs 30-11 30-13) In contrast to what occurs in the human disorder circulating erythrocytes contained relatively little uroporphyrin and coproporphyrin but much protoporphyrin [212] While most of the uroporphyrin and coproporphyrin in bone marrow and circulating erythrocytes was of type I isomer [212] the isomer type of the protoporphyrin has not yet been determined It is conceivable that the high protoporphyrin content of bovine red cells may be due in part to formation of protoporphyrin I If further studies should confirm this hypothesis it would be the only instance in which protoporphyrin of type I isomer has been demonstrated in nature It would further indicate that bovine erythrocytes must be capable of decarboxylating coproporphyrinogen I an enzymatic mechanism lacking in red cells of the other species studied

effect of splenectomy observed in some of the cases may be due to a reduction in the rate of hemolysis and hence in erythroid hyperplasia which results in reduced porphyrin formation [39]

HEREDITARY CONSIDERATIONS

*It was assumed by Carrod [241] and by Guenther [13] that erythropoietic porphyria is an inborn error of metabolism probably inherited* as a Mendelian recessive character The data presently available support this belief but they do not bear out the additional claim that the condition is more common in the male [13 242] In fact of the 41 cases listed in Table 30-1 24 were female and only 17 male As noted earlier the apparent male predominance in earlier statistics [185] was probably due to the erroneous inclusion of cases of porphyria cutanea tarda which occurs more frequently in males [16]

Of the 41 cases listed in Table 30-1 16 were observed in families with at least 2 cases [8 199 200 208 215 216 224-226] and in 2 instances 3 cases were reported in the same family all occurring in a single generation [208 215-217] In addition in 5 families 6 siblings of patients with erythropoietic porphyria were reported to have died in infancy exhibiting features which may have been porphyria (these are not included in Table 30-1) [8 191 195 203 213 214] Even without statistical analysis these data clearly show that erythropoietic porphyria not uncommonly occurs in siblings a finding which is contrary to a recent statement by Waldenström [16]

In 19 of the cases the family history was reported in sufficient detail to permit estimation of the number of unaffected siblings The 19 patients with porphyria were found to have 52 siblings who did not exhibit the disease *a ratio which closely approximates that predicted if the ab*normality is inherited as an autosomal recessive gene In 3 instances 2 out of 4 siblings were affected [199 200 224 225] whereas in 1 family only 1 out of 12 children had porphyria [218] In 3 of the reported families consanguinity of the parents was recorded [194 200 219]

The disease has never been observed in successive generations but reproduction is almost nil [15] The patient reported by Kench et al [197] gave birth to two apparently *normal* children one of whom is alive the other died shortly after a premature delivery During the first few days of life the living baby excreted considerable amounts of porphyrins which were believed to have been passively transferred through the placenta This is the only instance in which pregnancy has been reported in a patient with erythropoietic porphyria In addition the healthy wife of the famous case Petry had two spontaneous abortions each in the fourth month of pregnancy [243] The fetuses were carefully studied but no abnormalities of pigment metabolism were detected [243]

On the basis of these findings it appears probable that porphyric

patients are homozygous for the trait The sexes are equally affected The lack of increased porphyrin excretion in parents and in normal siblings of patients [217 225] suggests that individuals who are heterozygous for the trait do not exhibit the metabolic abnormality Thus the mode of inheritance of erythropoietic porphyria appears to be autosomal recessive

## CONGENITAL PORPHYRIA IN OTHER MAMMALS

**Cattle** Congenital porphyria has been observed in cattle in South Africa Denmark England and the United States [212 244–247] In all instances the disease was inherited as a simple Mendelian recessive characteristic Heterozygous animals are clinically and biochemically normal [212 246 247] Porphyric calves were obtained by breeding animals which were known to be heterozygous for the trait [212 248]

Afflicted animals exhibit photosensitivity of those areas of the skin which are not pigmented or covered with dark hair [212 248] The first case of bovine porphyria recognized in Denmark was discovered because the cow was not getting on at pasture but did well in the stable When the animals are slaughtered bones and teeth are found to be dark and to contain a large amount of porphyrin [212 244 246 248] The bovine form of congenital porphyria appears to have distinct similarities to the human disease Anemia reticulocytosis and splenomegaly may be present [212] but splenectomy performed in one porphyric cow failed to result in significant improvement [212] The spleen contained much porphyrin mostly uroporphyrin I the porphyrin concentration in the liver was insignificant [212 247] As in the human disease highest porphyrin concentrations were found in the bone marrow [212] and on fluorescence microscopic examination unstained bone marrow preparations exhibited intense porphyrin fluorescence which was most prominent in normoblastic nuclei On staining these cells exhibited abnormalities of nuclear structure similar to those described in human porphyria (Figs 30-11 30-13) In contrast to what occurs in the human disorder circulating erythrocytes contained relatively little uroporphyrin and coproporphyrin but much protoporphyrin [212] While most of the uroporphyrin and coproporphyrin in bone marrow and circulating erythrocytes was of type I isomer [212] the isomer type of the protoporphyrin has not yet been determined It is conceivable that the high protoporphyrin content of bovine red cells may be due in part to formation of protoporphyrin I If further studies should confirm this hypothesis it would be the only instance in which protoporphyrin of type I isomer has been demonstrated in nature It would further indicate that bovine erythrocytes must be capable of decarboxylating coproporphyrinogen I an enzymatic mechanism lacking in red cells of the other species studied

effect of splenectomy observed in some of the cases may be due to a reduction in the rate of hemolysis and hence in erythroid hyperplasia which results in reduced porphyrin formation [39]

HEREDITARY CONSIDERATIONS

It was assumed by Garrod [241] and by Guenther [13] that erythropoietic porphyria is an inborn error of metabolism probably inherited as a Mendelian recessive character The data presently available support this belief but they do not bear out the additional claim that the condition is more common in the male [13, 242] In fact, of the 41 cases listed in Table 30 1, 24 were female and only 17 male As noted earlier the apparent male predominance in earlier statistics [188] was probably due to the erroneous inclusion of cases of porphyria cutanea tarda which occurs more frequently in males [16]

Of the 41 cases listed in Table 30-1 16 were observed in families with at least 2 cases [8 199, 200 208 215, 216 224-226] and in 2 instances 3 cases were reported in the same family, all occurring in a single generation [208 215-217] In addition in 5 families 6 siblings of patients with erythropoietic porphyria were reported to have died in infancy exhibiting features which may have been porphyria (these are not included in Table 30-1) [8 191 195 203 213 214] Even without statistical analysis these data clearly show that erythropoietic porphyria not uncommonly occurs in siblings a finding which is contrary to a recent statement by Waldenstrom [16]

*In 19 of the cases the family history was reported in sufficient* detail to permit estimation of the number of unaffected siblings The 19 patients with porphyria were found to have 52 siblings who did not exhibit the disease a ratio which closely approximates that predicted if the abnormality is inherited as an autosomal recessive gene In 3 instances 2 out of 4 siblings were affected [199 200 224 225] whereas in 1 family only 1 out of 12 children had porphyria [218] In 3 of the reported families consanguinity of the parents was recorded [194 200, 219]

The disease has never been observed in successive generations but reproduction is almost nil [15] The patient reported by Kench et al [197] gave *birth to two apparently normal children one of whom is alive*, the other died shortly after a premature delivery During the first few days of life the living baby excreted considerable amounts of porphyrins which were believed to have been passively transferred through the placenta This is the only instance in which pregnancy has been reported in a patient with erythropoietic porphyria In addition the healthy wife of the famous case Petry had two spontaneous abortions each in the fourth month of pregnancy [243] The fetuses were carefully studied but no abnormalities of pigment metabolism were detected [243]

On the basis of these findings it appears probable that porphyric

## HEPATIC FORMS OF HUMAN PORPHYRIA

### INTRODUCTION AND CLASSIFICATION

Although in congenital erythropoietic porphyria the metabolic defect can be traced to the developing red cells in the bone marrow where an abnormality in heme biosynthesis accounts for the overproduction of porphyrins of type I isomer such a relationship cannot be demonstrated in the other and more frequently encountered forms of human porphyria Such patients show no hematologic disorder [18 123] and the porphyrin content of bone marrow and circulating erythrocytes is invariably normal [41 123] The only organ consistently exhibiting high concentrations of porphyrins or porphyrin precursors or both is the liver [18 28 41 55-59 128 185] On the basis of these observations it has been proposed to group the latter forms of porphyria together under the heading *hepatic porphyria* as contrasted with the erythropoietic form [18 39 41] This should not be considered as a final and unalterable classification it would be much preferable to describe each form of the disease in terms of the metabolic defect involved Since this stage of knowledge has not yet been reached it appears at least desirable to make a clear distinction between erythropoietic porphyria on one hand and the other less well-defined disorders of porphyrin metabolism on the other hand

In the hepatic forms of porphyria not only does the liver contain much porphyrin and many porphyrin precursors but impairment of hepatic function or outspoken liver disease is frequent [39 41] In fact in the photosensitive types of hepatic porphyria liver disease may be so severe as to make it difficult to decide whether the disturbance in porphyrin metabolism is the result of a genetically controlled defect or is a manifestation of advanced hepatic disease [16 38 42 128 137] This is particularly true of those patients in whom there is no evidence for a familial occurrence of the disorder [38 40 42 43 254 255] As mentioned earlier there is also reason to believe that a syndrome resembling acute porphyria may develop in supposedly normal persons as a result of addiction to sedatives of the sulfonal and Sedormid group [13 24 26-28 34 36 256] One may question whether such cases should not better be designated as *porphyrinuria* reserving the term *porphyria* for clearly inherited forms of the disorder However since in many instances it may be impossible to rule out with certainty a preexisting but unrecognized abnormality of pyrrole metabolism such a distinction would be arbitrary and might better be postponed until more is known of the basic defects involved

To this problem in deciding whether the metabolic disturbance is inherited or acquired is added the present confusion surrounding the classification of porphyria [16 17 39 137] Many attempts at a classification acceptable from all standpoints—clinical chemical genetic and

The urine of porphyric cattle contains much uroporphyrin I and smaller amounts of coproporphyrin I [*157, 212, 246, 248*]; in the feces this ratio is reversed [*157, 212*]. PBG is usually not demonstrable in the urine, nor could it be detected in the liver of an afflicted animal [*212, 247, 248*]. It is apparent that bovine congenital porphyria, like its human counterpart, is characterized by overproduction of porphyrins of type I isomer in cells of the erythroid series. The recent finding by Rimington's group [*180*] that incubation of PBG with hemolyzed erythrocytes obtained from porphyric cattle resulted in formation of both uroporphyrinogen I and uroporphyrinogen III suggests that the metabolic defect in the bovine disease may be similar to that in human porphyria.

**Pigs.** Congenital porphyria is also observed in pigs [*157, 246, 248, 249*], but in contrast to the human and bovine forms, the porcine disease is probably inherited as a Mendelian dominant trait [*157, 246*], although further studies on the precise mode of inheritance are necessary. Porphyric pigs excrete in the urine large amounts of uroporphyrin and smaller quantities of coproporphyrin and of porphyrins with seven, six, and five carboxyl groups; the feces contain mostly coproporphyrin [*249*]. Almost all the excreted porphyrins belong to the type I isomer series [*157*]; porphobilinogen is not demonstrable [*246*]. Affected pigs do not exhibit photosensitivity, and the diagnosis rests mainly on the discovery of red fluorescence in bones and teeth. Teeth of newborn pigs with porphyria contain considerable amounts of uroporphyrin I [*157, 246*], a finding which serves as a convenient mode of diagnosis during lifetime [*246*].

Little is known of the biochemical nature and the anatomic site of the metabolic defect. It remains to be shown whether the overproduction of porphyrins of type I isomer occurs in erythroid cells, as in the human and bovine forms of the disease, or whether the pigments are derived from other sites. Chemical and microscopic studies of bone marrow have not yet been reported, but With [*157*] observed high concentrations of porphyrins in the spleen and much smaller amounts in liver, kidneys, and blood. Pigs affected with the disease appear to show a considerable degree of fluctuation in the amounts of porphyrins produced [*157*]. In some instances where high concentrations of uroporphyrin were demonstrated in the teeth at birth, no evidence for a disturbance in porphyrin metabolism was detectable a few months later at autopsy [*157*]. This has been taken to indicate that the porcine form of porphyria may show a high degree of latency [*157*], a factor which renders genetic studies very difficult.

**Other Species.** Finally, it should be noted that in at least two species, the fox squirrel (*Sciurus niger*) and the touraco bird (*Musophagidae*), uroporphyrin is a physiologic end product of metabolism [*250–253*]. The metabolic origin of the pigment in these species is not known.

[16 39] These manifestations are characteristically intermittent in nature Acute attacks may last from several days to many months Between acute episodes periods of remission occur during which symp toms may be slight or even absent [18]

Neurologic manifestations are extremely variable They may involve peripheral nerves autonomic nervous system brain stem cranial nerves or cerebral function Extensive reviews of the neurologic and neuro-pathologic findings have recently been published [16 18 20 187 262 263] As noted earlier, the relationship of porphyrins and porphyrin precursors to the disturbances of the nervous system is not clear It is noteworthy that at autopsy the brain and the spinal cord of patients with porphyria usually fail to exhibit PBG or increased concentrations of porphyrins [41 187, 264]

Many of these patients have hypertension but this is not always present and at times the blood pressure may actually be low [265] Photosensitivity and increased mechanical fragility of the skin are lack ing The gastrointestinal manifestations frequently lead to weight loss and occasionally to severe emaciation while prolonged vomiting may cause oliguria and azotemia Death usually occurs from respiratory paralysis but uremia and cachexia may be contributing factors

In many instances the metabolic defect may be present in a latent form so that afflicted persons are entirely free of symptoms or exhibit only vague complaints such as dyspepsia or nervousness [14 16 39] In such patients careful analysis of the urine frequently reveals excretion of increased amounts of PBG or ALA or both [16 19 39 137] Latent porphyria may never become clinically manifest or acute attacks may occur at a later date In patients with latent porphyria acute attacks may be precipitated by the administration of various compounds notably barbiturates sulfonamides and anesthetics [14 20 39] This is however not an invariable sequence of events as the author has re peatedly observed patients with latent porphyria who underwent major surgery under general anesthesia *without* developing symptoms of porphyria

In most instances acute attacks unless terminating fatally are fol lowed by periods of latency during which only a careful search for ab normal urinary metabolites may reveal the presence of the metabolic disturbance It is not uncommon that during latency the conventional qualitative test for PBG [142] may become negative [16 39] and the diagnosis must be established by quantitative analysis for PBG and ALA [19]

*Chemical Findings*

The most characteristic finding in this type of porphyria is the urinary excretion of large amounts of PBG [14 266] As has been discussed PBG

pathogenetic—have provided little clarification [*18 16 23 39 41, 131, 182 257*] The reasons for this failure are twofold In the first place one and the same term has often been used to describe syndromes with widely varying symptomatology This is best exemplified by considering the different meanings which *porphyria cutanea tarda* has been given by different investigators The term as originally coined by Waldenstrom in 1937 [*14*] was used to describe patients in whom porphyrinuria and photosensitivity developed late in life in the absence of abdominal and neurologic symptoms Today, however, the same author distinguishes three or possibly four different types of *porphyria cutanea tarda* [*16*] while others [*18, 39 41*] still use the term in its original restricted sense In addition Rimington [*17*] states that "in this condition [porphyria cutanea tarda] abdominal and neurological manifestations may be the most prominent features while the skin may be insignificantly affected or indeed appear quite normal It is obvious that this lack of unanimity in terminology has handicapped the search for a satisfactory classification [*40*]

A second factor contributing to the present confusion was the discovery that in the same family a genetically controlled disturbance of porphyrin metabolism could give rise to clinical manifestations of widely varying nature, including abdominal neurologic or cutaneous symptoms [*137 258*] A purely descriptive classification of such a metabolic disturbance is obviously inadequate In the following discussion an attempt will be made to consider the various forms of hepatic porphyria as they appear in genetically homogeneous groups Such an approach meets with a number of limitations the most serious of which is the present ignorance regarding the precise nature of the metabolic defect

## SWEDISH TYPE OF PORPHYRIA

### *Clinical Manifestations*

The most extensive study of this type of familial porphyria was undertaken by Waldenstrom in 1937 [*14*] although many similar cases had been reported previously [*13*] The condition has been described as acute porphyria [*13 14 16*] or as intermittent acute porphyria [*18*]

Abdominal pain of moderate to severe degree and often colicky in nature is the initial and frequently the most prominent symptom The pain may be generalized or localized but the abdomen is usually soft, and tenderness is not marked Rebound tenderness is lacking X ray examination frequently reveals areas of intestinal distention proximal to areas of spasm [*259*] Constipation is usually marked and may give rise to confusion with bowel obstruction [*260 261*] Distention of the stomach and pernicious vomiting are at times outspoken Slight fever leukocytosis, and tachycardia may occur but are not universally present

[16 39] These manifestations are characteristically intermittent in nature Acute attacks may last from several days to many months Between acute episodes periods of remission occur during which symptoms may be slight or even absent [18]

Neurologic manifestations are extremely variable They may involve peripheral nerves autonomic nervous system brain stem cranial nerves or cerebral function Extensive reviews of the neurologic and neuropathologic findings have recently been published [16 18 20 187 262 263] As noted earlier, the relationship of porphyrins and porphyrin precursors to the disturbances of the nervous system is not clear It is noteworthy that at autopsy the brain and the spinal cord of patients with porphyria usually fail to exhibit PBG or increased concentrations of porphyrins [41 187 264]

Many of these patients have hypertension but this is not always present and at times the blood pressure may actually be low [265] Photosensitivity and increased mechanical fragility of the skin are lacking The gastrointestinal manifestations frequently lead to weight loss and occasionally to severe emaciation while prolonged vomiting may cause oliguria and azotemia Death usually occurs from respiratory paralysis but uremia and cachexia may be contributing factors

In many instances the metabolic defect may be present in a latent form so that afflicted persons are entirely free of symptoms or exhibit only vague complaints such as dyspepsia or nervousness [14 16 39] In such patients careful analysis of the urine frequently reveals excretion of increased amounts of PBG or ALA or both [16 19 39 137] Latent porphyria may never become clinically manifest or acute attacks may occur at a later date In patients with latent porphyria acute attacks may be precipitated by the administration of various compounds notably barbiturates sulfonamides and anesthetics [14 20 39] This is however not an invariable sequence of events as the author has repeatedly observed patients with latent porphyria who underwent major surgery under general anesthesia *without* developing symptoms of porphyria

In most instances acute attacks unless terminating fatally are followed by periods of latency during which only a careful search for abnormal urinary metabolites may reveal the presence of the metabolic disturbance It is not uncommon that during latency the conventional qualitative test for PBG [142] may become negative [16 39] and the diagnosis must be established by quantitative analysis for PBG and ALA [19]

*Chemical Findings*

The most characteristic finding in this type of porphyria is the urinary excretion of large amounts of PBG [14 266] As has been discussed PBG

*is an obligatory intermediate in the biosynthesis of porphyrins and of heme* and small amounts of it are normally excreted in the urine It is a colorless monopyrrolic chromogen giving an intense red color with Ehrlich s aldehyde (p dimethylaminobenzaldehyde) it has a strong absorption band at 560 mμ and a weaker one at 525 mμ [*123*] In contrast to the red compound obtained with urobilinogen the Ehrlich aldehyde compound of PBG is not extractable with chloroform [*123*] The concentration of PBG present in normal urine is too small to be detected with the conventional method [*143*] described by Watson and Schwartz [*142*] During acute attacks of porphyria however, the test is strongly positive and a positive result is usually but not invariably obtained during latency Estimation of PBG excretion during acute manifestations has yielded values ranging from less than 30 to 176 mg per liter urine [*137*] and from 50 to 170 mg per day [*37, 136*] During periods of latency these values may be much lower [*19 37 137*]

In addition to PBG the urine usually contains large amounts of ALA another obligatory intermediate in the biosynthesis of heme During an acute episode the urine may contain as much as 180 mg ALA per day [*37 136 137*] during remission the values may be much lower [*37, 137*]

Although the urinary excretion of large amounts of these two compounds is a typical feature of this form of porphyria, the urine may also contain other porphyrin precursors which give a negative Ehrlich reaction [*39 159–161 267*] Some of these chromogens are undoubtedly porphyrinogens or their partially oxidized derivatives porphomethene and porphodimethene (Fig 30 10) In the urine all these porphyrin precursors including PBG and possibly ALA can be converted to porphyrins [*121 144 162*] but the extent of this process and the nature of the porphyrins obtained depends largely on external factors such as pH exposure to light and air and presence of oxidizing substances [*121 122 128 144 268*] Freshly voided urine of patients with acute porphyria may contain little if any increase in uroporphyrin concentration [*122*] *porphyrins are formed only on standing or during the extraction procedure* This is probably why freshly passed urine frequently is of normal color but darkens on standing in light and air [*123*] It should be noted however that the color is not all due to porphyrins but in part reflects formation of porphobilin a brown amorphous oxidation product of PBG [*123*]

The many extensive studies which have been undertaken in an attempt to define more clearly the nature of the porphyrins present in the *urine of patients with acute porphyria or formed on standing* [*14 56–58 122 162 268–272*] have yielded seemingly controversial results One can probably reconcile these different findings by realizing that most of the isolated porphyrins are actually formed after the urine has been passed and thus are largely artifactual [*121 122*] There is little doubt that

uroporphyrin of both isomer types is present but the isomer ratio appears to be influenced by the conditions under which precursors are converted to porphyrins. A curious and as yet unexplained finding is that most of the porphyrins are present as metal complex, the metal probably being zinc [18, 172]. This is in contrast to erythropoietic porphyria, where uroporphyrin I is largely excreted in its free form.

Few data are available on porphyrin excretion in the stool. Uroporphyrin has been isolated, but the concentration does not appear to be

TABLE 30-3 UROPORPHYRIN CONCENTRATIONS IN LIVER AND URINE BEFORE AND AFTER CONVERSION OF PRECURSORS IN PATIENTS WITH THE SWEDISH TYPE OF PORPHYRIA

| Sex | Age | URO in liver µg/100 g wet weight | | URO in urine µg/100 ml | |
|---|---|---|---|---|---|
| | | *Before* | *After* | *Before* | *After* |
| F | 23 | 73 | 133 | 270 | 1 500 |
| M | 60 | 23 | 804 | 37 | 2 080 |
| M | 31 | Trace | 445 | 170 | 7 840 |
| M | 24 | 70 | 410 | 5 180 | 37 000 |
| F | 23 | | 1 600 | 113 | 15 800 |
| F | 67 | 18 | 143 | 595 | 1 475 |
| M | 49 | 31† | 528 | 305 | 3 980 |
| F | 27 | 47† | 131 | PBG present | |
| F‡ | 17 | 1 080† | 3 390 | PBG present | |

Liver brei and urine adjusted to pH 4 and heated for 15 min in boiling water bath
† Homogenate tested for PBG and found to be strongly positive
‡ Specimens stored in deep freeze for 18 months prior to analysis
Note URO = uroporphyrin
SOURCE R Schmid et al [41]

very high [39, 41, 58, 273]. Fecal excretion of coproporphyrin and protoporphyrin is little if at all increased [137].

With the exception of the liver, tissues obtained at autopsy usually fail to reveal increased porphyrin concentrations [14, 18, 28, 41, 55, 57, 58, 128]. In some cases uroporphyrin or coproporphyrin or both have been identified and isolated from hepatic tissue [41, 47, 58], but this is not possible in all instances [54]. More important is the demonstration that the liver regularly contains large amounts of PBG [41, 56, 57], while in other organs, including the spleen, muscle, and in bone marrow, this precursor has not been detected [41, 56]. Biopsy studies performed on a larger group of patients with acute or latent porphyria showed that fresh liver tissue contained only insignificant amounts of porphyrins, but that a marked increase in uroporphyrin concentration could be obtained after converting precursors to porphyrins (Table 30-3) [41].

These findings indicate that in the Swedish type of porphyria the principal metabolic abnormality is the presence and excretion not of porphyrins but of *porphyrin precursors* The large quantities of ALA PBG and other porphyrin precursors excreted in the urine are probably derived from the liver since this is the only organ in which they can be demonstrated It appears unlikely that these compounds could have reached the liver from other sites since injection of ALA and PBG does not lead to accumulation of these compounds in the liver of experimental animals [*146*]

*Possible Nature of the Metabolic Defect*

If the major biochemical defect in this form of porphyria is assumed to involve overproduction of ALA and PBG two possible mechanisms have to be considered which could give rise to this metabolic derangement It is conceivable that the biosynthesis of some heme-containing compound may be blocked and that increased pyrrole formation would result from an attempt by the liver to overcome this block [*110*] Alternatively the defect could be at the ALA level resulting either in increased formation of this compound or in interference with one or more of its metabolic pathways of disposition

Little formation is available to support the first of these two hypotheses As noted earlier the liver contains a number of heme proteins some of which appear to have a relatively rapid turnover The metabolism of these compounds has not been investigated in porphyria In experimental porphyria produced in animals by Sedormid inhibition of catalase synthesis in the liver has been demonstrated [*35*] but it is not known how this defect is related to the overproduction of porphyrins and of porphyrin precursors [*36*] In human porphyria the few determinations of liver catalase activity have failed to reveal a significant decrease in enzymatic activity [*56 177*] A further argument against the concept of a block in heme protein synthesis is the absence of significantly increased concentrations of coproporphyrin and protoporphyrin [*41*] Increased formation of these compounds would be expected if one of the final steps of heme biosynthesis were blocked (Fig 30 8)

The second hypothesis which assumes a defect of formation or metabolism of ALA is consistent with preliminary findings obtained in studying the pool size and disposition of ALA and PBG [*82 274*] The percentage of labeled ALA converted to urinary PBG was found to be several times greater in porphyria than in normal subjects (Fig 30 16) [*82*] This may indicate an adaptive increase in activity of ALA dehydrase in the liver but direct evidence for this has not yet been obtained Moreover the apparent dilution of administered ALA and of PBG suggested that in porphyria the pools of these compounds are increased (Table 30-4) If this is not simply because of altered permeability of the cells for ALA

it may indicate either an increase in formation of ALA or a decrease in one or more of the nonporphyrin pathways by which this compound can be metabolized (Fig 30 5) [82]

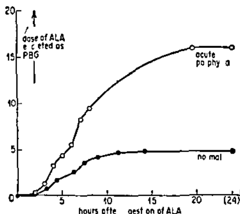



Fig 30-16 Conversion of δ-aminolevulinic acid (ALA) to porphobilinogen (PBG) by a normal man and by a patient with acute porphyria Both were given a dose of 1 gm ALA per 65 kg body weight Excretion of PBG is expressed as percentage of ALA administered (*By permission of J J Scott [8 ]*)

Direct support for this hypothesis is difficult to obtain It has been observed that in experimental Sedormid porphyria increased formation of porphyrins is associated with decreased purine synthesis [275] a finding which may suggest that an additional amount of ALA is channeled towards PBG production Similar studies in human porphyria have not yet been reported

TABLE 30-4 SPECIFIC DILUTION OF ADMINISTERED LABELED ALA BY ENDOGENOUS ALA AND PBG COMBINED

| *Subject* | *ALA type of label* | *Specific dilution capacity of ALA and PBG as μmole PBG/kg/day* |
|---|---|---|
| Normal | [1 4 C ] | 43 |
| Patient I acute porphyria (in remission) | [N ] | 237 |
| Patient II acute porphyria (in relapse) | [1 4 C ] | 334 |

Six millimoles of ALA was administered and the labeling of the excreted PBG was determined

SOURCE J J Scott [8 ]

In *R spheroides* Shemin and his coworkers [87] have recently obtained evidence for a heat labile inhibitor of ALA synthesis It was observed that extracts of *R spheroides* cells grown aerobically in the dark are far

less active for ALA synthesis than extracts obtained from cells grown anaerobically in the light More important still is the finding that formation of ALA is inhibited when extracts of aerobically dark grown cells are added to extracts from anaerobically light grown cells This suggests that at the step of ALA synthesis, the cell may possess a regulatory mechanism by which porphyrin and heme biosynthesis can be controlled [87] If it is permissible to extrapolate these findings to those in acute porphyria, one may speculate that patients with this disease may have a defect involving this regulatory mechanism [87] Such a disturbance *could be limited to the liver or it could be more extensive and include* for example, the central nervous system In the latter case a possible explanation would be provided for the protean nature of the neurologic *findings and the patchy anatomic lesions [121] Proof for these speculations* must of course await further investigations

As noted before increased excretion of ALA and PBG may be detected in asymptomatic carriers of the disease Furthermore acute attacks of porphyria may be followed by periods of complete symptomatic remission *During latency such patients usually continue to excrete abnormal* amounts of PBG and ALA [*19 137*] but at times the concentration of these metabolites in the urine may return to values scarcely above normal [*16 19 39*] This explains not only why during periods of latency patients may be asymptomatic but why it may be difficult to uncover their metabolic derangement The factors responsible for this fluctuating course are poorly understood although it is well known that barbiturates sulfonamides and related compounds may exert an aggravating effect [*14 20 39*]

It has also been noted that the age of onset of symptoms is usually at or shortly following puberty (Fig 30 17) [*16*] manifest porphyria is exceedingly rare before puberty [*39 276–278*] In children of afflicted kinships even the presence of latent porphyria is often difficult to detect before adolescence [*19*] Furthermore the death rate for porphyria is highest in young adults [*16*] These observations indicate that the metabolic defect even though it is inherited may not become clinically apparent until puberty and that symptomatic manifestations are most pronounced during the early part of the reproductive period

A possible relationship between porphyrin metabolism and gonadal activity is apparent but its nature is not known In female patients, periodic exacerbations of the disease are sometimes correlated with the menstrual cycle The author has followed a young woman in whom severe abdominal pain and intestinal obstruction regularly recurred during the premenstrual phase and terminated abruptly with the onset of vaginal flow For the last 4 years as menstruation has been suppressed by androgenic therapy the patient has remained *entirely free of symptoms* [*177*] The effect of pregnancy on porphyria is unpredictable [*279*] In

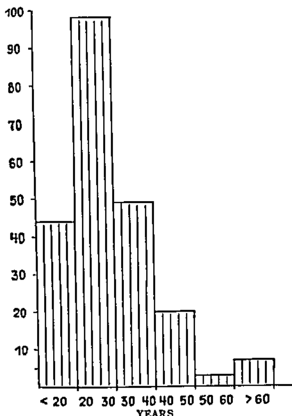



Fig 30 17 Age at first clinical manifestation of Swedish type of porphyria (*By permission of J Waldenstrom [16]*)

several instances relapses have occurred during or shortly following delivery [*18 39*]

*Heredity and Incidence*

Waldenstrom s extensive study in Sweden [*14 16 280*] has shown that a positive family history could definitely be established for 242 of 321 proved cases of porphyria One hundred and thirty seven cases belonged to a single family The three next largest families contained 14 12 and 9 porphyric members respectively On the basis of these findings it has been assumed that the metabolic defect is inherited as a Mendelian dominant trait [*14 16 280*] The frequency with which the disturbance remains asymptomatic and the fact that even genetically certain carriers may fail to exhibit an easily detectable increase in PBG excretion render genetic studies very difficult A representative pedigree from the Swedish study is given in Fig 30-18 The youngest generation comprised largely

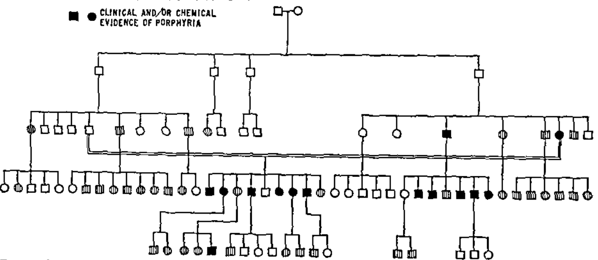



Fig 30-18 Pedigree of a family with the Swedish type of porphyria Urine of clinically normal individuals was examined with the semiquantitative method for porphobilinogen It is probable that with the advent of refined methods for estimation of urinary porphobilinogen and δ-aminolevulinic acid more cases of latent porphyria will be discovered This applies particularly to the fifth generation which is comprised largely of children [15] (*By permission of J Waldenström* [16])

of children cannot be adequately evaluated because quantitative determinations of PBG and ALA excretion have not yet been reported [19] The occurrence of the disease in several successive generations is a strong indication for its dominant mode of inheritance [16]

Familial occurrence of acute porphyria has been reported by other investigators Watson [39] studied 97 cases and Markovitz [263] collected from the literature 69 cases of acute porphyria many of which had a positive family history Acute attacks of porphyria were observed in two pairs of identical twins [16 281] In earlier studies marked predilection for the female sex was reported [14] Later reports however based on a larger amount of case material yielded a female to male ratio of 3 2 [16 39 263] Latent porphyria on the other hand appeared to be more frequent in males [39] This is consistent with the concept that the metabolic disturbance is inherited as a Mendelian dominant trait but more frequently produces symptoms in young adults of female sex

The incidence of the disease obviously is influenced by its occurrence in large families For Lapland for example Waldenstrom [16] has calculated an incidence of 1 1 000 largely because a single family with no less than 137 known porphyric persons lives in this northern province On the other hand for Sweden proper the incidence is about 1 5 100 000 Judging from the number of reported cases in the literature it would appear that the disease is more frequent in individuals with Scandinavian or English ancestry while in Negroes its occurrence is extremely rare [282 283]

*Recapitulation*

The Swedish type of hepatic porphyria is characterized by abdominal and neurologic symptoms which are frequently intermittent in nature Cutaneous manifestations are lacking The abnormality is believed to be inherited as a Mendelian dominant trait but afflicted individuals may be free of symptoms Qualitative methods of investigation may fail to reveal the metabolic derangement in asymptomatic persons who are genetically certain carriers

The defect almost certainly involves overproduction of the porphyrin precursors ALA and PBG Increased excretion of porphyrins is variable and may be absent

It has been pointed out [16 137] that acute porphyria and intermittent acute porphyria are misnomers for this condition for the disease is based on a genetically controlled metabolic disorder of chronic nature Furthermore the primary disturbance probably does not involve porphyrin metabolism but occurs at the level of pyrrole biosynthesis which is interrelated with the tricarboxylic acid cycle In future revisions of nomenclature these factors must be taken into consideration

## SOUTH AFRICAN TYPE OF PORPHYRIA

### *Clinical Manifestations*

Dean [*284*] and Dean and Barnes [*137, 258*] have studied a large group of porphyric patients in the white population of South Africa In 13 families a total of 236 porphyric persons were discovered all of whom could be traced back to a pair of early settlers who immigrated from Holland and married at the Cape of Good Hope in 1688 [*258*] Many of these patients exhibit cutaneous manifestations most pronounced on the hands and face The skin is sensitive to both sunlight and mechanical trauma, and abrades easily with occasional blister formation In some of the afflicted individuals skin sensitivity may be slight and intermittent Cutaneous manifestations are often the only symptoms in men who feel otherwise well throughout life In some members of this population acute attacks with abdominal pain and paralysis may occur they are frequently precipitated by barbiturates or anesthetics

In women skin sensitivity is usually slight but it may be more pronounced during pregnancy They are likely to develop recurrent attacks of abdominal pain which may simulate an acute surgical emergency These patients are usually emotionally unstable but the diagnosis of porphyria is seldom made before an acute attack of porphyria has occurred This is likely to happen at some time during adult life and is nearly always precipitated by drugs especially barbiturates During an acute episode abdominal pain becomes more severe peripheral and bulbar paresis may appear and respiratory paralysis may result in coma and death Associated with these manifestations is evidence of marked impairment of liver function and excretion of large amounts of PBG and porphyrins in the urine Acute attacks rarely occur before the age of 16 and are seldom observed after 50 photosensitivity is occasionally present at an earlier age [*285*] In many of these persons the disease remains latent throughout life or abdominal and cutaneous symptoms may be so mild as not to come to a physician s attention

### *Chemical Findings*

In this form of porphyria the characteristic finding is the continuous excretion of large amounts of coproporphyrin and protoporphyrin in the feces In normal persons upper limits of fecal porphyrin concentration are assumed to be 15 [*137*] to 20 μg [*17*] for coproporphyrin and 30 [*137*] to 45 μg [*17*] for protoporphyrin all expressed per gram of dry weight As seen in Table 30-5 in patients with South African porphyria the concentration of these porphyrins in the feces is greatly increased [*137 220*] Fecal excretion of porphyrins is elevated even though the patients may be in clinical remission or may have been asymptomatic all their lives

[137] Furthermore increased porphyrin concentration in the stool is demonstrable in asymptomatic children of patients with porphyria [220] As seen in Table 30 6 14 out of 35 such children under the age of 18 were found to have elevated values It is assumed that these children have latent porphyria which may become manifest during adult life [220]

TABLE 30 5 URINARY ALA AND PBG EXCRETION AND FECAL PORPHYRIN EXCRETION IN THE SOUTH AFRICAN TYPE OF PORPHYRIA

| Sex | Ag | Clinical status | Urine | | | Stool (dry) | |
|---|---|---|---|---|---|---|---|
| | | | PBG qualitative [14 ] | PBG mg/l | ALA mg/l | COPRO μg/gm | PROTO μg/gm |
| M | 28 | Acute attack | +++ | 194 | 219 | 830 | 1 400 |
| F | 4 | Acute attack | +++ | 99 | 45 | 5[illegible]0 | 574 |
| M | [illegible]9 | Acute attack | ++ | 40 | 41 | 805 | 810 |
| F | 35 | Acute attack | ++ | 138 | 81 | 6[illegible]3 | 805 |
| M | 29 | Acute attack | ++ | 31 | 30 | 670 | [illegible]85 |
| F | 43 | Mild attack | + | 25 | 26 | 6[illegible]0 | 1 240 |
| F | 4[illegible] | Recent attack | + | 20 | 8 | 345 | 568 |
| F | 35 | Recent attack | Neg | 6 | [illegible] | 6[illegible]3 | 39[illegible] |
| M | 23 | Recent attack | Trace | 24 | 81 | 580 | 830 |
| M | 28 | Recent attack | Neg | [illegible] | 7 | 270 | 508 |
| M | 5[illegible] | Mild pain | Neg | 3 | 3 | 363 | 266 |
| F | 39 | Mild pain | Neg | 8 | 5 | 477 | 930 |
| M | 23 | Mild pain | Neg | 6 | 11 | 630 | 760 |
| F | 35 | Latent | Neg | 2 | 2 | 1 [illegible]0 | 1 310 |
| M | 54 | Latent | Neg | 1 | 5 | 106 | 18[illegible] |
| F | | Latent | Neg | 0 | 1 | 70 | 165 |
| M | 29 | Latent | Neg | [illegible] | 3 | 414 | 490 |
| F | 33 | Latent | Neg | 1 | 1 | [illegible]43 | 2 000 |
| M | 49 | Latent | Neg | 1 | [illegible] | 159 | 131 |

Note COPRO PROTO = coproporphyrin protoporphyrin respectively
SOURCE G Dean and H D Barnes [13 ]

Occasionally photosensitivity is already present during adolescence [285] In such instances the finding of increased protoporphyrin excretion in the stool may help to differentiate this disorder from erythropoietic porphyria [220]

During clinical remission or in patients with latent porphyria the excretion of porphyrins in the urine may not be significantly increased [137] although exact estimation and analysis of the urinary pigments has not yet been reported During acute episodes urinary porphyrin excretion is usually much increased [137 258] More important is the finding that in patients with acute or subacute symptoms the urine

regularly contains large amounts of ALA and PBG (Table 30 5) while during remission, the excretion of these compounds may return to normal values [*137*] As a rule, the occurrence of abdominal and neurologic mani

TABLE 30 6 PORPHYRIN CONCENTRATION IN THE FECES OF ASYMPTOMATIC CHILDREN OF PARENTS WITH THE SOUTH AFRICAN TYPE OF PORPHYRIA

| *Group* | *No of children* | *Stool (dry weight)* | | | |
|---|---|---|---|---|---|
| | | *COPRO μg/gm* | | *PROTO μg/gm* | |
| | | *Mean* | *Range* | *Mean* | *Range* |
| Normal | 23 | 7 5 | 0–14 | 23 | 5–51 |
| Abnormal but asymptomatic | 14 | 52 | 16–158 | 116 | 51–212 |
| Control normal Bantu | 13 | 5 | 1–11 | 10 | 3–27 |

Note COPRO PROTO = coproporphyrin protoporphyrin respectively
SOURCE H D Barnes [*220*]

festations appears to be associated with urinary excretion of porphyrin precursors while increased fecal excretion of porphyrins is present at all times apparently unrelated to the presence or absence of clinical symptoms

*Comparison with Other Forms of Porphyria*

The high fecal excretion of coproporphyrin and protoporphyrin by white patients in South Africa clearly differentiates this form of porphyria from that studied by Waldenstrom in Sweden [*14*, *16*, *137*] In the latter group of patients fecal porphyrin excretion is either within normal limits or only moderately increased [*137*] In an earlier study it had been suggested that the absence of cutaneous symptoms in Sweden and their presence in male patients in South Africa may be related to increased exposure to sunlight and to mechanical trauma [*258*] In a more recent investigation however Dean et al [*137*] found Swedish patients whose symptomatology and excretory pattern resemble those seen in South Africa, in that cutaneous symptoms are prominent and the feces contain large amounts of coproporphyrin and protoporphyrin In South Africa on the other hand a group of patients of British stock, who did not have South African ancestry were found to have features similar to those in the large Swedish families in that cutaneous manifestations are absent and fecal porphyrin excretion is only slightly increased [*137*] Furthermore in Holland these authors studied two porphyric families, of which one resembles the South African cases and the other has many features of the disturbance present in the Swedish families [*137*] These observations seem to indicate that the differences in clinical and bio

chemical features are not due to environmental factors but are the result of different genetic patterns

Although the number of different genes responsible for abnormalities in pyrrole and porphyrin metabolism is not known it is conceivable that some of the cases reported as chronic porphyria [12 13 287] mixed porphyria [39 41 286] or porphyria cutanea tarda [17 33] may be related to the type of porphyria present in the white population of South Africa In two patients with mixed porphyria exhibiting cutaneous abdominal and neurologic manifestations liver tissue obtained by biopsy revealed high concentrations of uroporphyrin [41] At a later date when photosensitivity was no longer present but abdominal pain was marked a repeat biopsy of the liver in one of these patients revealed little preformed porphyrin but large amounts of porphyrin precursors [41] Moreover these patients excreted large quantities of PBG and uroporphyrin in the urine at all times The data presently available do not permit a decision as to whether the disease of these patients is etiologically related to that in South Africa or whether it represents a separate genetic defect

Similar difficulties are encountered in comparing the South African disease with that which Holti and Tio [17 288] have described under the term of porphyria cutanea tarda In their patients increased fecal excretion of coproporphyrin and protoporphyrin is a constant feature but acute attacks with outspoken neurologic manifestations are infrequent and only during such episodes are small amounts of PBG occasionally detectable in the urine In the South African families on the other hand acute attacks involving the nervous system and at times terminating fatally occur frequently and are invariably associated with excretion of large amounts of PBG and ALA in the urine

*Inheritance*

The studies by Dean and Barnes [137 258] indicate that in the white population of South Africa porphyria is inherited as a Mendelian dominant trait The 236 cases discovered occurred in 13 families all of which had a common ancestry The disease could be traced through six successive generations

A representative family tree is given in Fig 30-19 [258] The progenitor of this family was born in 1814 and had 478 descendants many of whom were still alive at the time of this study and could be traced As seen in Table 30-7 there were 60 porphyric patients among the 125 descendants with a porphyric parent excluding children under 18 years of age in the sixth generation In all cases only one parent had porphyria In the second generation 5 of the 10 members had porphyria in the third generation 16 out of 37 in the fourth generation 32 out of 59 in the fifth generation 7 out of the 19 descendants who were over 18 years of age had porphyria

regularly contains large amounts of ALA and PBG (Table 30-5), while during remission, the excretion of these compounds may return to normal values [137] As a rule, the occurrence of abdominal and neurologic mani

TABLE 30 6 PORPHYRIN CONCENTRATION IN THE FECES OF ASYMPTOMATIC CHILDREN OF PARENTS WITH THE SOUTH AFRICAN TYPE OF PORPHYRIA

| Group | No of children | Stool (dry weight) | | | |
|---|---|---|---|---|---|
| | | COPRO μg/gm | | PROTO μg/gm | |
| | | Mean | Range | Mean | Range |
| Normal | 23 | 7 5 | 0–14 | 23 | 5-51 |
| Abnormal but asymptomatic | 14 | 52 | 16–158 | 116 | 51 212 |
| Control normal Bantu | 13 | 5 | 1–11 | 10 | 3 27 |

Note COPRO PROTO = coproporphyrin protoporphyrin respectively
SOURCE H D Barnes [[illegible]0]

festations appears to be associated with urinary excretion of porphyrin precursors while increased fecal excretion of porphyrins is present at all times, apparently unrelated to the presence or absence of clinical symptoms

*Comparison with Other Forms of Porphyria*

The high fecal excretion of coproporphyrin and protoporphyrin by white patients in South Africa clearly differentiates this form of porphyria from that studied by Waldenstrom in Sweden [14 16, 137] In the latter group of patients fecal porphyrin excretion is either within normal limits or only moderately increased [137] In an earlier study it had been suggested that the absence of cutaneous symptoms in Sweden and their presence in male patients in South Africa may be related to increased exposure to sunlight and to mechanical trauma [258] In a more recent investigation however Dean et al [137] found Swedish patients whose symptomatology and excretory pattern resemble those seen in South Africa in that cutaneous symptoms are prominent and the feces contain large amounts of coproporphyrin and protoporphyrin In South Africa on the other hand a group of patients of British stock who did not have South African ancestry were found to have features similar to those in the large Swedish families in that cutaneous manifestations are absent and fecal porphyrin excretion is only slightly increased [137] Furthermore in Holland these authors studied two porphyric families of which one resembles the South African cases and the other has many features of the disturbance present in the Swedish families [137] These observations seem to indicate that the differences in clinical and bio

In the sixth generation all descendants were under 18 years of age in a later study many of these children were found to have increased fecal porphyrin excretion [220] Thus 48 per cent of the adults with one porphyric parent inherited the disturbance 24 males and 36 females

Studies of the other 12 porphyric families revealed a similar pattern of inheritance In the total group of patients 121 were male and 115 were female Of the adults with one porphyric parent 53 4 per cent exhibited the disease This conforms with the requirements for a Mendelian autosomal dominant type of inheritance

## OTHER FORMS OF FAMILIAL PORPHYRIA

Holti and his coworkers [17] recently described a porphyric family in Britain in which 10 out of 19 members studied 4 males and 6 females exhibited abnormally high porphyrin excretion The disturbance was

TABLE 30-7 NUMBER OF MEMBERS IN EACH GENERATION TOTAL ADULT CHILDREN OF AFFECTED PARENTS AND NUMBER AND PERCENTAGE OF AFFECTED CHILDREN IN A FAMILY WITH THE SOUTH AFRICAN TYPE OF PORPHYRIA*

| Generation | Total members | Adult children of affected parent | | | Affected | | | Percentage affected |
|---|---|---|---|---|---|---|---|---|
| | | No | M | F | No | M | F | |
| I | 1 | | | | | | | |
| II | 10 | 10 | [illegible] | 5 | 5 | 3 | 2 | [illegible]0 |
| III | 6[illegible] | 37 | 16 | [illegible]1 | 1[illegible] | [illegible] | [illegible] | [illegible] |
| IV | 173 | 59 | 17 | 4[illegible] | 3[illegible] | 10 | [illegible] | 54 2 |
| V | [illegible]1 | 19 | [illegible] | 16 | | | [illegible] | 36 8 |
| VI | 3[illegible] | | | | | | | |
| Total | 4[illegible]9 | 125 | 41 | 84 | 60 | 24 | 3[illegible] | 48 |

* The fifth generation contains many juveniles and young adults and the sixth generation only children Since fecal excretion of porphyrins has not yet been reported for these members under 18 years of age these determinations may raise the percentage of affected persons

SOURCE ( Dean et al [ 58]

present in four successive generations Clear evidence for autosomal dominant inheritance was established (Fig 30-20)

The family member most severely affected was a young woman who had been subject to constipation since early childhood At various times he experienced attacks of abdominal colic anorexia nausea giddiness postural hypotension peripheral neuropathy and headaches During pregnancy her symptoms were more pronounced and bullous skin lesions appeared for the first time 1 hr after delivery Subsequently areas of

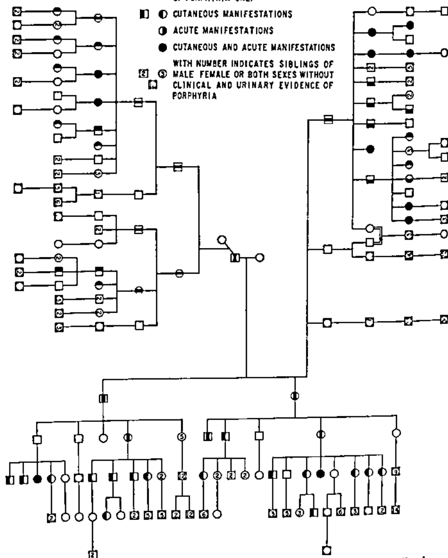



Fig 30-19 Pedigree of a family with the South African type of porphyria The diagnosis was established on the basis of clinical manifestations and urinary analysis but fecal examinations for porphyrins have not yet been reported for this family The last generation is comprised largely of children under 18 years of age many of whom may exhibit increased porphyrin excretion in the feces [~ 0] (*By permission of G Dean and H D Barnes* [2~8])

relationship between urinary and biliary excretion of porphyrins [59] In such patients during remission large amounts of coproporphyrin and protoporphyrin are excreted in the feces On the other hand during periods of jaundice and liver failure fecal porphyrin excretion is reduced in favor of increased urinary excretion of uroporphyrin and at times of small amounts of PBG During these latter episodes photosensitivity and abdominal colic are present and neuropathic effects may be observed It is apparent that in the porphyric family described above [17] such a reciprocity between urinary and biliary excretion of porphyrins was not evident since urinary porphyrin excretion was either within normal limits or was only slightly increased Furthermore none of the afflicted members exhibited jaundice or liver failure even though cutaneous abdominal and neurologic manifestations were present

It is difficult to compare this familial form of porphyria with cases of pure porphyria cutanea tarda [18 39 41] with mixed porphyria [39 41 286] or with cutaneous porphyria in the Bantu race [42] in whom the role of genetic factors has not been clearly established These latter patients often excrete large amounts of uroporphyrin or coproporphyrin or both in the urine and increased urinary porphyrin excretion frequently persists during periods of clinical remission [39 41] The porphyria in the British family also differs from the hereditary porphyria in the white population of South Africa in that in the latter acute abdominal and neurologic manifestations associated with excretion of large amounts of PBG are much more prominent Thus the available evidence suggests that the genetically controlled disturbance present in this British family may differ from both the South African *and* the Swedish types of porphyria and that it may be distinguished from the various cases of chronic or cutaneous porphyria in which a familial occurrence cannot be established

Similar uncertainty in classification exists in considering other families in whom an apparently inherited abnormality in porphyrin metabolism has been observed Tio Tiong Hoo [288] studied nine members of a Dutch family (five females four males) all of whom exhibited photodermatitis with blister formation The first evidence of cutaneous manifestations occurred between 16 and 30 years of age abdominal and neurologic symptoms were consistently absent Large amounts of coproporphyrin and protoporphyrin were excreted in the feces whereas the urine contained only intermittently small amounts of uroporphyrin and never contained PBG

Calvy et al [*86*] reported a family of mixed porphyria in which eight members had bizarre neurologic manifestations in addition to cutaneous lesions Finally Berger and Goldberg [*292*] discovered a Swiss family in which four asymptomatic members excreted large quantities of coproporphyrin in urine and stool while uroporphyrin and PBG were not detectable The abnormality was most pronounced in a 10-year old

skin habitually exposed to sunlight exhibited marked fragility but direct light sensitivity was not detectable Laboratory studies indicated a slight microcytic hypochromic anemia and mild impairment of hepatic function but jaundice or evidence of liver failure was not present

Of the other 9 members who exhibited chemical evidence of porphyria only 1 was light sensitive and 6 had noticed an unusual degree of fragility of exposed skin while 3 had no cutaneous manifestations At one time or other, however all 9 had experienced mild gastrointestinal and

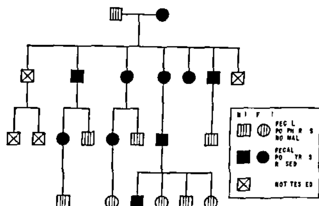



Fig 30-20 Pedigree showing the dominant autosomal inheritance of porphyria in the family described by Holti et al [17] (*By permission of G Holti et al [17]*)

neurologic symptoms Chemically the characteristic finding in this family was a marked increase in fecal excretion of coproporphyrin and protoporphyrin Elevated values were present both in patients who exhibited porphyric manifestations and in those who were in temporary remission On the other hand urinary porphyrin excretion was within normal limits or was only slightly increased The nature and isomer type of the porphyrins in the urine has not been reported nor are quantitative data available on the excretion of PBG and ALA except that the semiquantitative test for PBG [142] gave at times a slightly positive reaction

Rimington [17 128] classifies these patients as having ' porphyria cutanea tarda and Waldenstrom [16] and Rimington [289] have proposed the name protocoproporphyria hereditaria While this latter term may be applicable to the disturbance in this particular family in that the major biochemical abnormality appears to consist in the continuous excretion of large amounts of coproporphyrin and protoporphyrin in the feces different excretory patterns are observed in other patients with similar clinical manifestations On the ba i of study of a few isolated and apparently nonfamilial cases of porphyria which were described under the term of porphyria cutanea tarda [69 290 291] or of chronic porphyria [287] Rimington has postulated the existence of a reciprocal

diagnosed disease (Table 30 8) Its occurrence in more than one child of the same family and the ratio of 1 3 for affected to unaffected siblings of both sexes suggest that the abnormality is inherited as a Mendelian autosomal recessive trait The penetrance of the abnormal gene appears to be complete since *formes frustes* are not observed Parents and unaffected siblings of patients fail to exhibit abnormalities in porphyrin excretion

2 Impressive progress has been made in uncovering the nature of the metabolic defect and the problem appears to be within sight of solution The major derangement undoubtedly consists of an enzymatic defect in the developing red blood cells which leads to increased formation of porphyrins of type I isomer

3 For the *other forms of inherited porphyria* (Table 30 8) the situation is less clear Different genetic types can be recognized but on symptomatic grounds alone a separation is not always possible Cases of latent porphyria or patients exhibiting only minimal symptoms are frequently encountered Moreover in many instances the metabolic abnormality cannot be shown to be genetically controlled but may be acquired or may possibly represent a new mutation Further studies are necessary to clarify the existing confusion

4 In each patient or genetically homogeneous group of patients with porphyria the excretory pattern of porphyrin precursors and of porphyrins in urine and feces should be studied both during symptoms and during clinical remission These investigations should be performed with generally accepted methods and should include as many asymptomatic blood relatives as possible This information would provide an indispensable basis for comparison of the different clinical forms of porphyria A most promising beginning of such a study has been made by Dean and Barnes [137] The investigations have permitted a clear separation of the types of porphyria present in the Swedish and in the South African families More important still is the elucidation of the nature of the metabolic defects involved and it is here that the most promising field for future studies lies

## ADDENDUM

An additional case of congenital erythropoietic porphyria has recently been published [293] A female infant presumably of British stock was noticed to pass pink staining meconium and urine during the first days after birth Hemolytic anemia splenomegaly hypertrichosis and erythrodontia were present Splenectomy performed at the age of 6 months resulted in marked improvement of the hemolytic process and in reduced porphyrin excretion in the urine Erythropoietic cells in the bone marrow and peripheral blood exhibited the characteristic mor

boy coproporphyrinuria of a lesser degree was present in both parents and in an aunt

In all these apparently inherited disorders of pyrrole and porphyrin metabolism the number of different abnormal genes is unknown and little is understood of the nature of the metabolic defects Moreover it is difficult to determine to what extent clinical and chemical manifestations of the inherited abnormality may be modified by superimposed acquired disturbances such as alcoholism and malnutrition or by environmental factors such as excessive exposure to sunlight The important role of the liver as a major site of the metabolic derangement is generally recognized [*16 39 41 128 132*] The association of cutaneous photosensitivity with the presence of preformed porphyrins both in the liver and in the excreta [*41*] is well documented It has also been noted however that in many patients who excrete large amounts of porphyrins cutaneous manifestations may be absent or the skin may merely exhibit excessive fragility to mechanical trauma but little direct sensitivity to sunlight The reason for this is not understood Moreover it is not clear why in some family groups like those reported by Holti [*17*] and by Calvy [*286*] patients may exhibit abdominal colic and neurologic disturbances in the absence of significantly increased PBG excretion Although there is no evidence to suggest that this compound exerts a toxic effect on the gastrointestinal tract or the nervous system acute attacks are invariably associated with increased excretion of PBG and ALA in both the Swedish and the South African type of hepatic porphyria

It has recently been suggested [*132*] that the appearance of preformed porphyrins in the liver and their excretion in bile and urine may reflect an inherited or acquired defect in those cellular enzyme systems which normally prevent porphyrinogens from being oxidized to porphyrins Such a decrease in cellular reducing potential could result in increased conversion of porphyrinogens to porphyrins Since the latter cannot be drawn back into the biosynthetic pathway (Figs 30 8 to 30 10) they escape from the cells and are excreted While such a mechanism would account for increased formation of preformed porphyrins in the liver it would not explain an overproduction of ALA and PBG For instance in a patient with mixed porphyria [*41*] at a time of marked photosensitivity the liver exhibited a high concentration of preformed porphyrins while later when only abdominal and neurologic symptoms were present the liver contained mainly porphyrin precursors It is possible that in such instances more than one metabolic defect is involved

## SUMMARY

1 Among the various forms of inherited disorders of porphyrin metabolism *erythropoietic porphyria* stands out as a well defined and easily

phologic and fluorescence-microscopic abnormalities in addition needle like structures believed to consist of precipitated or crystalline por phyrins were present in some of the abnormal normoblasts and non nucleated red cells The patient has one healthy brother

Additional familial cases of erythropoietic porphyria have recently been published [*294 295*] According to recent reports from Turkey [*296 297*] occurrence of a cutaneous form of porphyria has been observed in some 400 persons who consumed government-distributed wheat con taminated with various fungicides Preliminary information indicates that the wheat contained hexachlorobenzene and several organic mercury and copper compounds

A syndrome of cachexia photo ensitivity bullous skin eruptions hyperpigmentation and hypertrichosis developed in individuals who had consumed contaminated wheat 1 month to 3 years prior to the onset of manifestations Urine and feces contained large amounts of uro- and coproporphyrins but increased excretion of urinary porphobilinogen was not observed Abdominal and neurologic manifestations were absent but there was functional and anatomic evidence of liver disease

The syndrome appeared to be more frequent in children under 15 years of age but was al o found in adults of various racial background who had been exposed to contaminated cereals

The preliminary reports suggest that this syndrome may represent an *acquired* form of porphyrinuria and may perhaps be similar to the por phyrinuria seen in the Bantus of South Africa

## BIBLIOGRAPHY


1 Baumstark F Zwei pathologische Harnfarb toffe Pflugers Arch ges Physiol **9** 568 1874

2 MacMunn C A Observations on the colouring matters of the so-called bile of invertebrates on those of the bile of vertebrates and on some unu ual urine pig ments Proc Roy Soc **35** 370 1883

3 Salkowski E Ueber Vorkommen und Nachweis des Haematoporphyrins im Harn Ztschr physiol Chem **15** 286 1891

4 Hammarsten O Ueber Haematoporphyrin im Harn Skandinav Arch Physiol **3** 319 1892

5 Garrod A E On haematoporphyrin as a urinary pigment in disease J Path & Bact **1** 187 1893

6 Stokvis B J Zur Pathogene e der Haematophorphyrie Ztschr klin Med **28** 1 1895

7 Saillet De l'uro pectrine (ou urohématoporphyrine normale) et de sa transforma tion en hémochromogène sans fer Rev méd Paris **16** 54 1896

8 Anderson M T Hydroa aestivale in two brothers complicated with the presen e of haematoporphyrin in the urine Brit J Dermat **10** 1 1898

9 Nebelthau F Beitrag zur Lehre von Haematoporphyrin des Harnes Ztschr physiol Chem **27** 324 1899

10 Hoppe-Seyler F *Medizin -chem Unters chu g* Tuebingen 1871

11 Nencki M and Sieber N Ueber das Haematoporphyrin Arch exper Path u Pharmakol **24** 430 1888

12 Guentl er H Die Haematoporphyria Deutsch Arch klin Med **105** 89 1911

TABLE 30 8 SUMMARY OF THE DIFFERENT TYPES OF HEREDITARY HUMAN PORPHYRIA

| *Type* | *Synonyms* | *Clinical manifestations* | *Clinical findings* | *Inheritance* |
|---|---|---|---|---|
| Prophyria erythropoietica | Congenital photosensitive porphyria | Photosensitivity hemolytic anemia with plenomegaly | Large amounts of uroporphyrin I and coproporphyrin I in bone marrow erythrocytes and urine ALA and I BG in liver and urine | Recessive no abortive forms |
| Swedish type | Intermittent acute porphyria porphyria hepatica | Abdominal colic hypertension central nervous system involvement acute attacks frequent photosensitivity absent | | *Dominant many cases remain clinically latent* |
| South African type | Porphyria cutanea tarda mixed porphyria porphyria hepatica | Cutaneous abdominal and neurologic acute attacks frequent in female patients | Copro- and *protoporphyrin in liver* and *feces during attacks* ALA I BG *and porphyrins in* urine | Dominant many cases remain clinically latent |
| Other types | Porphyria cutanea tarda mixed porphyria porphyria hepatica | Cutaneous abdominal and *neuro*logic acute attacks rare | *Increased* excretion of fecal porphy*rins* urinary excretion of porphy*rins* and porphyrin precursors variable | Probably dominant variable degrees of latency |

43 Tio T H Leijnse B Jarrett A and Rimington C Acquired porphyria from a liver tumour Clin Sc 16 517 1957

44 Watson C J Some studies of nature and clinical significance of porphobilinogen A M A Arch Int Med 93 643 1954

45 With T K and Petersen H C A. Symptomatic uroporphyrinuria Lancet 2 1148 1954

46 Rimington C Haem pigments and porphyrins Ann Rev Biochem 26 561 1957

47 Vannotti A *Porphyrins Their Biological and Chemical Importance* Hilger and Watts Ltd London 1954

48 Bénard H Hémoglobine et Pigments Apparentés Masson et Cie Paris 1949

49 Zoja L Sur quelques pigments de certaines urines et specialement sur la presence dans celles-ci de l hématoporphyrine et de l uroérythrine Arch ital biol 19 425 1893

50 Robitschek W Haematoporphyria congenita Ztschr klin Med 101 540 1925

51 Hegler C Fraenkel E and Schumm O Zur Lehre von der Haematoporphyria congenita Deutsch med Wchnschr 39 842 1913

52 Liebig H Ueber die experimentelle Bleihaematoporphyrie Arch exper Pathol u Pharmakol 126 16 1927

53 Fischer H Hilmer H Lindner F and Puetzer B Zur Kenntnis der natuerlichen Porphyrine Chemische Befunde bei einem Fall von Porphyrinurie (Petry) Ztschr physiol Chem 150 44 1925

54 Waldenstrom J Some observations on acute porphyria and other conditions with a change in the excretion of porphyrins Acta med scandinav 83 281 1934

55 Derrien E and Benôit C Notes et observations sur les urines et sur quelques organes d une femmes morte en crise de porphyrie aigue Arch Soc sc méd biol Montpellier et Languedoc 10 456 1929

56 Gray C H Acute porphyria A M A Arch Int Med 85 459 1950

57 Prunty F T G Acute porphyria Investigations on pathology of porphyrins and identification of excretion of uroporphyrin I Arch Int Med 77 623 1946

58 Watson C J Schwartz S and Hawkinson V Studies of the uroporphyrins II Further studies of the porphyrins of the urine feces bile and liver in cases of porphyria with particular reference to a Waldenstrom type porphyrin behaving as an entity on the Tswett column J Biol Chem 157 345 1945

59 MacGregor A G Nicholas R E H and Rimington C Porphyria cutanea tarda A M A Arch Int Med 90 483 1952

60 Drabkin D L Independent biosynthesis of different heme chromoproteins Cytochrome C in various tissues Proc Soc Exper Biol & Med 76 5 7 1951

61 Theorell H Beznak M Bonnichsen R Paul K O and Akeson A On the distribution of injected radio-active iron in guinea pigs and its rate of appearance in some hemoproteins and ferritins Acta chem scand nav 5 445 1951

62 Shemin D and Rittenberg D The biological utilization of glycine for the synthesis of the protoporphyrin of hemoglobin J Biol Chem 166 621 1946

63 Shemin D and Rittenberg D The life span of the human red blood cell J Biol Chem 166 627 1946

64 London I M Shemin D West R and Rittenberg D Heme synthesis and red blood cell dynamics in normal humans and in subjects with polycythemia vera sickle-cell anemia and pernicious anemia J Biol Chem 179 463 1949

65 Gray C H Neuberger A and Sneath P H A Studies in congenital porphyria 2 Incorporation of $^{15}N$ in the stercobilin in the normal and in the porphyric Biochem J 47 8[illegible] 1950

66 Shemin D London I M and Rittenberg D The in vitro synthesis of heme from glycine by the nucleated red blood cell J Biol Chem 173 799 1948

67 Dresel E I B and Falk J E Studies on the biosynthesis of blood pigments 1 Haem synthesis in haemolyzed erythrocytes of chicken blood Biochem J 56 150 1954

68 London I M Shemin D and Rittenberg D Synthesis of heme in vitro by the immature non nucleated mammalian erythrocyte J Biol Chem 183 749 1950

13 Guenther H In *Handbuch der Krankheiten des Blutes und der blutbildenden Organe* edited by A Schittenhelm vol 2 Springer Berlin 1925
14 Waldenstrom J Studien ueber Porphyrie Acta med scandinav Suppl 82 1937
15 Schmid R Schwartz S and Sundberg D Erythropoietic (congenital) porphyria a rare abnormality of the normoblasts Blood 10 416 1955
16 Waldenstrom J The porphyrias as inborn errors of metabolism Am J Med 22 758 1957
17 Holti G Rimington C Tate B C and Thomas G An investigation of porphyria cutanea tarda Quart J Med 27 1 1958
18 Watson C J In *Diseases of Metabolism* 3d ed Saunders Philadelphia 1953
19 Haeger B Urinary δ-aminolaevulic acid and porphobilinogen in different types of porphyria Lancet 2 606 1958
20 Waldenstrom J Neurological symptoms caused by so-called acute prophyria Acta psychiat et neurol 14 375 1939
21 Ellinger A and Riesser O Zur Kenntnis des im Harn nach Trionalvergiftung auftretenden Porphyrins Ztschr physiol Chem 48 1 1916
22 Watson C J In *Oxford Medicine* vol IV chap 9-A p 251 Oxford New York 1951
23 Micheli F and Dominici G Ueber zwei Falle von familiarer Prophyrie mit letalem Au gang Deutsch Arch klin Med 171 154 1931
24 Duesberg R Toxische Prophyrie Munchen med Wchnschr 79 1821 1932
25 Nakarai Ueber Hamatoporphyrinurie Deutsch Arch klin Med 58 165 1897
26 Stokvis B J Cited in Garrod A E *Inborn Errors of Metabolism* 2d ed Oxford London 1923
27 Taylor A E and Sailer J A fatal case of sulphonal poisoning Contrib Penn Univ William Pepper Lab 1900
28 Emminger E Fluoreszenz mikroskopische Untersuchungen bei einem Fall von Schlafmittelvergiftung Klin Wchnschr 12 1840 1933
29 Neubauer O Haematoporphyrin und Sulfonalvergiftung Arch exper Path 43 456 1900
30 Perutz A Ueber Hydroa aestivale und vacciniforme Arch Dermat u Syph 124 531 1917
31 *Kast A and Weiss Th Zur Kenntnis der Haematoporphyrinurie* Berlin klin Wchnschr 33 621 1896
32 Fischer H and Duesberg R Ueber Porphyrine bei klinischer und experimenteller Porphyrie Arch exper Path u Pharmakol 166 95 1932
33 Waldenstrom J and Wendt S Tierexperimentelle Studien ueber den Porphyrinstoffwechsel Ztschr physiol Chem 259 157 1939
34 Schmid R and Schwartz S Experimental porphyria III Hepatic type produced by Sedormid Proc Soc Exper Biol & Med 81 685 1952
35 Schmid R Figen J F and Schwartz S Experimental porphyria IV Studies of liver catalase and other heme enzymes in Sedormid porphyria J Biol Chem 217 263 1955
36 Schmid R and Schwartz S Studies of some liver heme proteins and porphyrins in experimental Sedormid porphyria in *Porphyrin Biosynthesis and Metabolism* Ciba Foundation Churchill London 1955
37 Stich W Ueber einen neuen biochemischen Unterschied zwischen humaner akuter Porphyrie und experimenteller Porphyria Klin Wchnschr 36 386 1958
38 Brunsting L A Observations on porphyria cutanea tarda Arch Dermat u Syph 70 551 1954
39 Watson C J The porphyrias in *Advances in Internal Medicine* vol 6 Year Book Publishers Inc Chicago 1954
40 Bolgert M Canivet J and LeSourd M La Porphyrie cutanée de l adulte Étude de neuf cas et description Semaine hôp Paris 29 1587 1953
41 Schmid R Schwartz S and Watson C J Porphyrin content of bone marrow and liver in the various forms of porphyria A M A Arch Int Med 93 167 1954
42 Barnes H D Porphyria in the Bantu races on the Witwatersrand South African M J 29 781 1955

95 Larsen E G and Orten J M Studies of porphyrin biosynth e is in the nucle ated avian erythrocyte Abstract 69C American Chemical Society 1 8th Meet ing Minneapolis September 1955
96 Dresel E I B and Falk J E Studies on the biosynthesis of blood pigments ? Haem and porphyrin formation in intact chicken erythrocytes Biochem J 63 72 1956
97 Granick S Enzymatic conversion of δ-aminolevulinic acid to porph ol ilinogen Science 120 1105 1954
98 Schmid R and Shemin D The enzymatic formation of porphobilinogen from δ-aminolevulinic acid and its conversion to protoporph yrin J Am Chem Soc 77 506 1955
99 Gibson K D Neuberger A and Scott J J The purification and properties of δ-aminolaevulinic acid dehydrase Biochem J 61 618 1955
100 Gibson K D Some properties of δ-aminolaevulinic acid d hydrase in *Po phyrin Biosynthesis and Metabolism* Ciba Foundation Churchill London 1955
101 Cookson G H and Rimington C Porphobilinogen chemical constitution Nature London 171 875 1953
10? Westall R G Isolation of porphobilinogen from the urine of a patient with acute porphyria Nature London 170 614 195?
103 Dresel E I B and Falk J E Studies on the biosynthesis of blood pigments 3 Haem and porphyrin formation from δ-aminolaevulinic acid and from por phobilinogen in haemolyzed chicken erythrocytes Biochem J 63 80 1956
104 Bogorad L Intermediates in the bio ynthesis of porphyrins from porphol i linogen Science 121 8 8 1955
105 Granick S and Mauzerall D Porphyrin biosynthesis in erythrocytes II Enzymes converting δ-aminolevulinic acid to coproporphyrinogen J Biol Chem 232 119 1958
106 Iodice A A Richert D V and Schulman M P Copper content of purified δ aminolevulinic acid dehydrase Fed Proc 17 248 1958
107 Anderson R L and Tove S B Effect of copper deficiency on synthesis of haem Nature London 182 315 1958
108 Falk J E Dresel E I B and Rimington C Porphobilinogen as a porphyrin precursor and interconversion of porphyrins in a tissue system Nature London 172 29? 1953
109 Bogorad L and Granick S The enzymatic synthesis of porphyrins from porphobilinogen Proc Nat Acad Sc 39 1176 1953
110 Schwartz S Porphyrins and porphyrin precursors in human and e perimental porphyria Fed Proc 14 717 1955
111 Dresel E I B The role of some porphyrins and porphyrin precursors in the biosynthesis of haem in *Porphyrin Biosynthesis and Metabolism* Ciba Foundation Churchill London 1955
112 Neve R A Labbe R F and Aldrich R A Reduced uroporphyrin III in the biosynthe is of heme J Am Chem Soc 78 691 1956
113 Mauzerall D and Granick S Porphyrin bio ynthe is in erythrocytes III Uroporphyrinogen and its decarboxyla e J Biol Chem 232 1141 1958
114 Hoare D S and Heath H Intermediates in the bio ynthesi of porphyrin from porphobilinogen by *Rhodop udo o as sph oi les* Nature London 181 159? 19 8
115 Townley P M and Neilands J B The iron and porphyrin metabolism of *Micrococc s lysodeikticus* J Biol Chem 224 695 19?
116 Bogorad L The enzymatic synthesis of porphyr ns from porphobilinogen I Uroporphyrin I J Biol Ch m 233 501 1958
117 Bogorad L The enzymatic synthesis of porphyrins from porphobil nogen II Uropo phyrin III J Biol Chem 233 510 19 8
118 Bogorad L The enzymatic synthesis of porphyrins from porphobilinogen III Uroporphyrinogens as intermediates J Biol Chem 233 516 1958
119 Fi her H and Orth H *D e Ch m e des I y rols* Akademische Verlagsgesell l ft m b H Leipzig 1937
120 Rimington C The biosynthesis of haemoglobin Brit M J, 2 189 1956

69 Muir H M and Neuberger A The biogenesis of porphyrins The distribution of $^{15}$N in the ring system Biochem J 45 163 1949
70 Shemin D The succinate-glycine cycle the role of δ-aminolevulinic acid in porphyrin synthesis in *Porphyrin Biosynthesis and Metabolism* Ciba Foundation Churchill London 1955
71 Muir H M and Neuberger A The biogenesis of porphyrins 2 The origin of the methene carbon atoms Biochem J 47 97 1950
72 Radin N S Rittenberg D and Shemin D The role of glycine in the biosynthesis of heme J Biol Chem 184 745 1950
73 Grinstein M Kamen M D and Moore C V Observation on the utilization of glycine in the biosynthesis of hemoglobin J Biol Chem 174 767 1948
74 Shemin D and Kumin S The mechanism of porphyrin formation The formation of a succinyl intermediate from succinate J Biol Chem 198 827 1952
75 Wriston J C Jr Lack L and Shemin D The mechanism of porphyrin formation Further evidence on the relationship of the citric acid cycle and porphyrin formation J Biol Chem 215 603 1955
76 Shemin D and Wittenberg J The mechanisms of porphyrin formation The role of the tricarboxylic acid cycle J Biol Chem 192 315 1951
77 Wittenberg J and Shemin D The location in protoporphyrin of the carbon atoms derived from the α-carbon atom of glycine J Biol Chem 185 103 1950
78 Shemin D and Russell C S Delta aminolevulinic acid its role in the biosynthesis of porphyrins and purines J Am Chem Soc 75 4873 1953
79 Shemin D Russell C S and Abramsky T The succinate-glycine cycle I The mechanism of pyrrole synthesis J Biol Chem 215 613 1955
80 Neuberger A and Scott J J Aminolaevulinic acid and porphyrin biosynthesis Nature London 172 1093 1953
81 Dresel E I B and Falk J E Conversion of δ-aminolaevulinic acid to porphobilinogen in a tissue system Nature London 172 1185 1953
82 Scott J J The metabolism of δ aminolaevulinic acid in *Porphyrin Biosynthesis and Metabolism* Ciba Foundation Churchill London 1955
83 Laver W G Neuberger A and Udenfriend S Initial stages in the biosynthesis of porphyrins 1 The formation of δ-aminolaevulinic acid by particles obtained from chicken erythrocytes Biochem J 70 4 1958
84 Gibson K D Laver W G and Neuberger A Initial stages in the biosynthesis of porphyrins 2 The formation of δ-aminolaevulinic acid from glycine and succinyl-coenzyme A by particles from chicken erythrocytes Biochem J 70 71 1958
85 Granick S Porphyrin biosynthesis in erythrocytes I Formation of δ-amino levulinic acid in erythrocyte J Biol Chem 232 1101 1958
86 Sano S Inoue S Tanabe Y Sumiya C and Koike S Significance of mitochondria for porphyrin and heme biosynthesis Science 129 275 1959
87 Kikuchi G Kumar A Talmage P and Shemin D The enzymatic synthesis of δ-aminolevulinic acid J Biol Chem 233 1214 1958
88 Gibson K D Biosynthesis of δ-aminolaevulinic acid by extracts of *Rhodopseudomonas spheroides* Biochim et biophys acta 28 451 1958
89 Sawyer E and Smith R A δ-Aminolaevulinate synthesis in *Rhodopseudomonas spheroides* *Bacteriological Proceedings* p 111 Society of American Bacteriologists Baltimore 1958
90 Schulman J P and Richert D A Heme synthesis in vitamin $B_6$ and pantothenic acid deficiencies J Biol Chem 226 181 1957
91 Lascelles J Synthesis of porphyrins by cell suspensions of *Tetrahymena vorax* effect of members of the vitamin B group Biochem J 66 65 1957
92 Brown E G The relationship of the tricarboxylic acid cycle to the synthesis of δ-aminolaevulinic acid in avian erythrocyte preparations Biochem J 70 313 1958
93 Wintrobe M M Factors and mechanisms in the production of red corpuscles Harvey Lect 45 87 1949–1950
94 du Vigneaud V Kuchinskas E J and Horvath A L-Penicillamine and rat liver transaminase activity Arch Biochem & Biophys 69 130 1957

148 Schwartz S *Clinical Aspects of Porphyrin Metabolism* Veterans Administration Tech Bull T B 10-94 1953
149 Zieve L Hill E Schwartz S and Watson C J Normal limits of urinary coproporphyrin excretion determined by an improved method J Lab Clin Med 41 663 1953
150 Welıky I and Shemin D Metabolism of α amino β ketoadipic acid Fed Proc 16 268 1957
151 Comfort A Moore H and Weatherall M Normal human urinary porphyrins Biochem J 58 177 1954
152 Watson C J Hawkinson V Schwartz S and Sutherland D Studies of coproporphyrin I The per diem excretion and isomer distribution of coproporphyrin in normal human urine J Clin Invest 28 447 1949
153 Hoffbauer F W Watson C J and Schwartz S Urinary and fecal coproporphyrin excretion in rats III Excretion of injected coproporphyrin Proc Soc Exper Biol & Med 83 238 1953
154 Lockwood W H and Bloomfield B Uroporphyrins III Crystalline uroporphyrin from normal human urine Australian J Exper Biol & Med 32 733 1954
155 Nicholas R E H and Rimington C Qualitative analysis of the porphyrins by partition chromatography Scandinav J Clin Lab Invest 1 12 1949
156 With T K and Petersen H C A Symptomatic uroporphyrinuria Lancet 2 1148 1954
157 With T K Personal communication
158 Watson C J Concerning the naturally occurring porphyrins V Porphyrins of the feces J Clin Invest 16 383 1937
159 Herbert F K The coproporphyrin precursor of human urine and another pigment formed from a chromogen Biochem J 69 10P 1958
160 Comfort A and Weatherall M Urinary porphyrins in lead treated rabbits Biochem J 54 217 1953
161 Watson C J Pimenta de Mello R Schwartz S Hawkinson V E and Bossenmaier I Porphyrin chromogens or precursors in urine blood bile and feces J Lab Clin Med 37 831 1951
162 Brockman P E and Gray C H Studies on porphobilinogen Biochem J 54 22 1953
163 Schwartz S and Wikoff H M The relation of erythrocyte coproporphyrin and protoporphyrin to erythropoiesis J Biol Chem 194 563 1952
164 Schmid R Schwartz S and Watson C J Porphyrins in the bone marrow and circulating erythrocytes in experimental anemia Proc Soc Exper Biol & Med 75 705 1950
165 Dobriner K and Rhoads C P The porphyrins in health and disease Physiol Rev 20 416 1940
166 Hausmann W and Haxthausen H *Die Lichterkrankungen der Haut* Urban & Schwarzenberg Berlin Munich and Vienna 19 9
167 Fischer H and Zerweck W Ueber den Harnfarbstoff bei norm len und pathologischen Verhältnissen und seine lichtsensibilisierende Wirkung Zugleich einige Beitrage zur Kenntnis der Porphyrinurie Ztschr physiol Chem 137 1 6 19 4
168 Meyer Betz F Untersuchungen ueber die b ol gi che (phot dynami che) Wirkung d s Haematoporphyrins und anderer D rivate des Blut und Gallenfarbstoffes Deut ch Arch klin Med 62 4 6 1913
169 Stra ıch C B *Photo en tization* Am J Dis Child 40 800 1930
170 Blum H F and Hardgrave I F Spectral region of photo en itivity in hydroa aestiv l seu vacciniforme with porphyrinuria Proc Soc Exper Biol & Med 34 613 1936
171 Blum H F and Pace N Studies of photosen itizat on by prophyrin Brit J Derm t & Syph 49 465 1937
172 Lemberg R and Legge J W *Hematin Compo ds a d Bile Pig ents* Inter ci n e Publ hers Inc New York 19
173 Fisch r H Ueber die Giftigkeit d e n ıt ıh ıeren le Wirkung das spektro-

121 Gray C H In *Biochemical Disorders in Human Disease* Academic Press Inc New York 1957

122 Cookson G H and Rimington C Porphobilinogen Biochem J 57 476 1954

123 Waldenstrom J and Vahlquist B Studien ueber die Entstehung der roten Harnpigmente (Uroporphyrin and Porphobilin) bei der akuten Porphyrie aus ihrer farblosen Vorstufe (Porphobilinogen) Ztschr physiol Chem 260 189 1939

124 Booij H L and Rimington C Effect of pre-heating on porphyrin synthesis by red cells Biochem J 65 4P 1957

125 Lockwood W H and Rimington C Purification of an enzyme converting porphobilinogen to uroporphyrin Biochem J 67 8P 1957

126 Shemin D Abramsky T and Russell C S The synthesis of protoporphyrin from δ-aminolevulinic acid in a cell free extract J Am Chem Soc 76 1204 1954

127 Dresel E I B and Falk J E Studies on the biosynthesis of blood pigments 5 Intermediates in haem biosynthesis Biochem J 63 388 1956

128 Rimington C In *Chemical Pathology of the Nervous System* Pergamon Press New York London in press [ed H Himwich]

129 Granick S and Mauzerall D Enzymic formation of protoporphyrin from coproporphyrinogen III Fed Proc 17 233 1958

130 Rimington C and Booij H L Porphyrin biosynthesis in human red cells Biochem J 65 3P 1958

131 Lascelles J The formation of porphyrins by photosynthetic bacteria in *Porphyrin Biosynthesis and Metabolism* Ciba Foundation Churchill London 1955

132 Heikel T Lockwood W H and Rimington C Formation of non-enzymic haem Nature London 182 313 1958

133 Krueger R C Melnick I and Klein J R Formation of heme by broken-cell preparations of duck erythrocytes Arch Biochem & Biophys 64 30 1956

134 Goldberg A Ashenbrucker H Cartwright G E and Wintrobe M M Studies on the biosynthesis of heme in vitro by avian erythrocytes Blood 11 821 1956

135 Wintrobe M M *Clinical Hematology* 4th ed Lea & Febiger Philadelphia 1956

136 Mauzerall D and Granick S The occurrence and determination of δ-amino levulinic acid and porphobilinogen in urine J Biol Chem 219 435 1956

137 Dean G and Barnes H D Porphyria in Sweden and South Africa South African M J 33 274 1959

138 Berlin N I Neuberger A and Scott J J The metabolism of δ-aminolevulinic acid 2 Normal pathways studied with the aid of C Biochem J 64 90 1956

139 Haeger B Increased content of a δ-aminolaevulinic acid like substance in urine from workers in lead industry Scandinav J Clin & Lab Invest 9 211 1957

140 Berlin N I Neuberger A and Scott J J The metabolism of δ aminolaevulinic acid 1 Normal pathways studied with the aid of $^{15}N$ Biochem J 64 80 1956

141 Granick S and Van den Schrieck H G Porphobilinogen and δ aminolevulinic acid in acute porphyria Proc Soc Exper Biol & Med 88 270 1955

142 Watson C J and Schwartz S A simple test for urinary porphobilinogen Proc Soc Exper Biol & Med 47 393 1941

143 Hammond R L and Welcker M L Porphobilinogen tests on a thousand miscellaneous patients in a search for false positive reactions J Lab Clin Med 33 1254 1948

144 Watson C J Some studies of nature and clinical significance of porphobilinogen A M A Arch Int Med 93 643 1954

145 Goldberg A and Rimington C Experimentally produced porphyria in animals Proc Roy Soc London s B 143 257 1955

146 Goldberg A Fate of porphobilinogen administered enterally or parenterally in the rat Biochem J 69 37 1955

147 Schwartz S Keprios M and Schmid R Experimental porphyria II Type produced by lead phenylhydrazine and light Proc Soc Exper Biol & Med 79 463 1952

or congenital porphyria with hemolytic anemia I Clinical and fundamental studies before and after splenectomy Bl x 1 6 (S) 1951
204 Meineri A Sindrome cutanea a tipo di epidermoli i bullo a a di idroa vacciniforme con porfiria Dermo ifilografo 6 383 1931
205 Ottolenghi Lodigiani F and Serchi C Le porfirine ed il loro metabolismo nella porfiria congenita Gior ital dermat sif 89 187 1948
206 Taussig L R Hypersensitivity of the skin to light California & West Med 39 301 1933
207 DeMarval L and Fons R Concomitancia entre una porfirinuria congénita en ictericia hemolitica Esplanectomia Arch argent pediat 5 2 0 1934
208 Hernando T La porphyrie ces manifestations dig tives cutanées et oculaires Biol méd Paris 36 293 1938
209 Leachey C H Dobriner K and Strain W H Hydroa estivale in congenital porphyria New York J Med 38 1 1938
210 London I M West R Shemin D and Rittenberg D Porphyrin formation and hemoglobin metabolism in congenital porphyria J Biol Chem 184 365 1950
211 Dobriner K Strain W H Guild H and Localio S A The excretion of porphyrins in congenital porphyria J Clin Invest 17 761 1938
212 Watson C J Perman V Spurrell F A Hoyt H H and Schwartz S Some studies of the comparative biology of human and bovine porphyria erythropoietica Tr A Am Physicians 71 196 1958
213 Dunsky I Freeman S and Gibson S Porphyria and porphyrinuria Report of a case review of porphyrin metabolism with a study of congenital porphyria Am J Dis Child 74 305 194
214 Rosenthal I M Lipton L L and Asrow G Effect of splenectomy on porphyria erythropoietica Pediatrics 15 663 1955
215 May F Bloch Michel H Poncet Guaret and Tournier P Porphyrie familiale (maladie de Gunther) Bull Soc méd hôp Paris 64 340 1948
216 Bolgert M Canivet J and Le Sourd L Trois cas de porphyrie cutanée congenitale (maladie de Gunther) dans la même fratrie Bull So franç dermat et syph 59 233 1952
217 Canivet J and Pelhard Con idere M Étude de l hemolyse dans deux cas de porphyrie congenitale Rev franç étud clin biol 3 7 1958
218 Cafetti A Luciti a tippo epidermolisi bullosa con porfiria Gior ital dermat sif 89 187 1948
219 Findlay G H and Barnes H D Congenital porphyria hydroa aestivale and hypertrichosis in a South African Bantu Lancet 2 846 1950
220 Barnes H D Porphyria in South Africa the faecal excretion of porphyrin South African M J 32 680 1958
221 Menagh F and Reyner C E Hydroa aestivale congenital porphyria A M A Arch Dermat & Syph 63 518 1951
222 Zuelzer W W and Kaplan E Personal communi ation
223 Lozzan M Porfiria congenita con iperemol si Acta paed lat matina (Parma) 6 935 1953
224 Weremowicz I Congenital porphyria with erythrodontia splenomegaly and anemia in two sisters Polski Tygo lnik Lekarski 9 550 585 1954
225 Town end Coles W F and Barnes H D Erythropoietic (congenital) porphyria Two ca es in Su l ne e sibling Lancet 2 1 195
226 Baxter B Erythropoietic (congenital) porphyria Central African J Med 4 148 1958
227 St h W Die kong nitale Porphyrie eine erythropathische haemolyti che Anamie (Porphyrocyto e) Schweiz med Wchnschr 41 101 19 8
228 Grin t in M Aldrich R A Hawkinson V and Watson C J An isotopic stud of porphyrin and hemoglobin metabol m in a case of porphyria J Biol Ch 179 383 1949
229 Gray C H Muir H and Neuberger A Stud es in congenital porphyria 3 The incorporation of N into the haem and glycine of haemoglobin Biochem J 47 54 1950

skopische Verhalten der naturlichen Porphyrine Ztschr physiol Chem 97 109 1916
174 Schwartz S In discussion of Scott J J (ref 82)
175 Feldberg W and Talesnik J Reduction of tissue histamine by compound 48/80 J Physiol 120 550 1953
176 Gray C H In discussion of Scott J J (ref 82)
177 Schmid R Personal observations
178 Jarrett A Rimington C and Willoughby D A δ Aminolaevulic acid and porphyria Lancet 1 125 1956
179 Pimenta de Mello R Effect of Rose Bengal and ultraviolet light on porphyrin excretion in rabbits Proc Soc Exper Biol & Med 72 292 1949
180 Pimenta de Mello R Effect of light on urinary coproporphyrin excretion in lead poisoned rabbits Proc Soc Exper Biol & Med 76 823 1951
181 Weatherall M Drugs and porphyria metabolism Pharmacol Rev 6 133 1954
182 Schreus H T and Carrie C Untersuchungen zum Gallenfarbstoffwechsel Klin Wchnschr 13 1670 1934
183 Reitlinger K and Klee P Zur biologischen Wirkung der Porphyrine Arch exper Path u Pharmakol 127 277 1928
184 Goldberg A Paton W D M and Thompson J W Pharmacology of the porphyrins and porphobilinogen Brit J Pharmacol 9 91 1954
185 Goldberg A and Rimington C Fate of porphobilinogen in the rat Relation to acute porphyria in man Lancet 2 172 1954
186 Bingel A *Die Bedeutung der Porphyrine fur die Pathogenese gewisser neurologischer Krankheitsbilder* Ztschr ges Neurol Psychiat 158 79 1937
187 Gibson J B and Goldberg A The neuropathology of acute porphyria J Path & Bact 71 495 1956
188 Turner W J and Obermayer M E Studies on Porphyria II A case of porphyria with epidermolysis bullosa hypertrichosis and melanosis Arch Dermat & Syph 37 549 1938
189 Radaeli F Contributo alla conoscenza dell hydroa vacciniforme di Bazin Gior ital mal vener 52 93 1911
190 Cappelli J Caso singolare di hydroa vacciniforme con ematoporfirinuria ed ipertricosi Gior ital mal vener 55 481 1914
191 Mackey L and Garrod A E On congenital porphyrinuria associated with hydroa aestivale and pink teeth Quart J Med 15 319 1922
192 Garrod A E Congenital porphyria a postscript Quart J Med (N S) 5 473 1936
193 Gray C H and Neuberger A Effect of splenectomy in a case of congenital porphyria Lancet 1 851 1952
194 Gray A M H Haematoporphyria congenita with hydroa aestivale Proc Roy Soc Med 17 43 1924
195 Sato A and Takahashi N A new form of congenital hematoporphyria oligochromemia porphyrinuria (megalosplenica congenita) Am J Dis Child 32 325 1926
196 Ashby H T Haematoporphyria congenita its association with hydroa vacciniforme and pigmentation of the teeth Quart J Med 19 375 1926
197 Kench J D Langley F A and Wilkinson J F Biochemical and pathological studies of congenital porphyria Quart J Med (N S) 22 285 1955
198 Schmidt La Baume F Hydroa aestivale Klin Wchnschr 6 827 1926
199 Kitagawa K Ueber Haematoporphyria congenita Hans Gunther und ihre experimentelle Untersuchung Jap J Dermat Urol 27 43 1927
200 Matsuoka K Ueber Haematoporphyria congenita Jap J Dermat Urol 28 38 1928
201 Marcozzi A Epidermolisi bullosa distrofica con ematoporfirinuria ed alterazione endocrino-simpatica (eritrodontia) Arch ital dermat sif 4 555 1929
202 Borst M and Koenigsdoerffer H *Untersuchungen ueber Porphyrie mit besonderer Beruecksichtigung der Porphyria congenita* S Hurzel Leipzig 1929
203 Aldrich R A Hawkinson V Grinstein M and Watson C J Photo en itive

5J Mason V R Courville C and Ziskind E The porphyrins in human disease Medicine 12 355 1933
(0 Calvy G L Porphyria A consideration in surgical diagnosis Surg Gynec & Obst 90 716 1950
?61 Watson C J Varco R L and Schmid R An unusual case of acute porphyria with volvulus and gangrene of the cecum Am J Med 22 980 1957
26? Denny Brown D and Sciarra D Changes in the nervous system in acute porphyria Brain 68 1 1945
?63 Markovitz M Acute intermittent porphyria a report of five cases and a review of the literature Ann Int Med 41 1170 1954
64 Kluever H In *Cereb al Mechanism in Behavior* Wiley New York 1951
26? Nesbitt S Acute porphyria J A M A 124 286 1944
?66 Sachs P Ein Fall von akuter Porphyrie mit hochgradiger Muskelatrophie Klin Wchnschr 10 11?3 1931
67 Raine D N An ether soluble precursor of coproporphyrin in urine Biochem J 47 XIV 1950
?68 Gibson Q H and Harrison D C A note on the urinary uroporphyrin in acute porphyria Biochem J 46 154 1950
269 Grinstein M Schwartz S and Watson C J Studies of the uroporphyrins I The purification of uroporphyrin I and the nature of Waldenström s uroporphyrin as isolated from porphyria material J Biol Chem 157 3?3 1945
?70 Nicholas R E H and Rimington C Studies on the Waldenstrom Porphyrin of acute porphyria urines Biochem J 55 109 1953
271 With T K Preparation of crystalline porphyrin esters from porphyria urines and normal human urine Scandinav J Clin Lab Invest 10 29? 1958
?? Fischer H and Libowitzky K Auftreten von Uro bzw Koproporphyrin I bei akuter Porphyrie Ztschr physiol Chem 241 ? 0 1936
273 Fischer H Über das Kotporphyrin Ztschr physiol Chem 97 148 1916
274 Gray C H and Scott J J The effect of haemorrhage on the incorporation of ($\alpha$-¹ C) glycine into stercobilin Biochem J 69 2?P 1958
275 Labbe R F Talman E L and Aldrich R A Porphyrin metabolism II Uric acid excretion in experimental porphyria Biochim et biophys acta 15 590 1954
276 Aldrich R A Labbe R F and Talman E L A review of porphyrin metabolism with special reference to childhood Am J M Sc 230 675 1955
277 Kark R M Clinical aspects of the major porphyrinopathies M Clin North America 39 11 1955
278 Lysaught J N and McCleery J M Acute intermittent porphyria Report of a case in an infant aged eight months with discussion of porphyrin metabolism J Ped at 46 55? 1955
?79 Vine S Shaffer H M Pauley G and Margolis F J A review of the relation hip between pregnancy and porphyria and presentation of a case Ann Int Med 47 834 1957
?80 Waldenström J Studies on the incidence and heredity of acute porphyria in Sweden Acta genet 6 1?? 1956
281 Kelloe L I Rudenky H and Reynolds W W Acute intermittent porphyria in identical twins Ann Int Med 47 131 1957
?8 Curtis A J and Gilberti J J Acute porphyria in a Negro New York J Med 50 1066 1950
283 Wiggins C A Fatal case of acute porphyria in a Negro Brit M J 2 866 1950
?84 Dean G Porphyria Brit M J 2 1?91 1953
8. Barnes H D Frootko J and Parnell J I Unusually early manifestations of porphyria cutanea tarda South African M J 31 34? 1957
?86 Caley C L Jaruzewski E J and Carroll H H Porphyria In al observa t n and a family vignette Ann Int Med 34 76? 1951
8? C y H Rimington C and Thomson S A case of chronic porphyria associated with recurrent jaundice Quart J Med 17 123 1948
88 To T H Beschouwingen over de Porphyria cutanea tarda Thesis University of Amsterdam 1956

230 Gray C H Isotope studies in porphyria Brit M Bull 8 229 1952
231 Rosenthal I M Personal communication
232 Hausmann W Die sensibilisierende Wirkung des Haematoporphyrins Biochem Ztschr 30 276 1910
233 London I M West R Shemin D and Rittenberg D On the origin of bile pigment in normal man J Biol Chem 184 351 1950
234 Gray C H The Bile Pigments Methuen London 1953
235 London I M Metabolism of hemoglobin and of bile pigment Bull New York Acad Med 30 509 1954
236 Rimington C and Miles P A A study of the porphyrins excreted in the urine by a case of congenital porphyria Biochem J 50 202 1951
237 Fischer H and Hofmann H J Ueber die Konstitution des Uro und Muschelschalenporphyrins Ztschr phy iol Chem 246 15 1937
238 Watson C J Porphyrin metaboli m in the anemias A M A Arch Int Med 99 323 1957
239 MacRae E K and Rosenthal I M Enzyme studies of hereditary acute and congenital porphyrias Records of the Genetics Society of America 28 84 1959
240 Thorell B Cellular formation of intermediates during haemoglobin synthesis in Porphyrin Biosynthesis and Metabolism Ciba Foundation Churchill London 1955
241 Garrod A E Inborn Errors of Metabolism 2d ed Henry Frowde London 19 3
242 Rimington C In Modern Trends in Blood Diseases Butterworth London 1955
243 Fraenkel E Experimentelles ueber Haematoporphyrie Virchow s Arch path Anat 248 125 1924
244 Fourie I J J The occurrence of congenital porphyrinuria (pink tooth) in cattle in South Africa (Swaziland) Onderstepoort J Vet Sc 2 535 1936
245 Rimington C Some ca es of congenital porphyrinuria in cattle chemical studies upon the living animals and post mortem material Onderstepoort J Vet Sc 7 567 1936
246 Joergensen S K and With T K Congenital porphyria in swine and cattle in Denmark Nature London 176 156 1955
247 Amoroso E C Loosmore R M Rimington C and Tooth B E Congenital porphyria in bovines first living cases in Britain Nature London 180 230 1957
248 Rimington C Haems and porphyrins in health and disease Acta med scandinav 143 161 177 1952
249 Clare N Y and Stephens E H Congenital porphyria in pigs Nature London 153 252 1944
250 Turner W J Studies on porphyria I Observations on the fox squirrel (Sciurus niger) J Biol Chem 118 519 1937
251 Rimington C A reinvestigation of turacin the copper porphyrin pigment of certain birds belonging to the Musophagidae Proc Roy Soc London s B 127 106 1939
252 Watson C J and Berg M Studies of the Uroporphyrins IV Reexamination of a crucial Waldenstrom porphyrin J Biol Chem 214 537 1955
253 With T K Pure unequivocal uroporphyrin III Simplified method of preparation from turaco feathers Scandinav J Clin Lab Invest 9 398 1957
254 Canivet J and Rimington C A chromatographic study of uroporphyrins from cases of cutaneous acute and congenital porphyria Biochem J 55 867 1953
255 Tappeiner S and Tirschek H Das Syndrom der aktinischtraumatischen bulloesen Porphyrindermatose Arch Dermat u Syph 196 65 1953
256 Harbitz F Hematoporphyrinuria as an independent disease ( hematoporphyria ) and as a symptom of liver di ease and intoxications Arch Int Med 33 632 1924
257 Schreus H T and Carrié C Zur Physiologie und Pathophysiologie der Porphyrin Ausscheidung Klin Wchnschr 12 745 1933
258 Dean G and Barnes H D The inheritance of porphyria Brit M J 2 89 1955

Part Nine

# Diseases Manifest Primarily in the Blood and the Blood forming Tissues

289 Rimington C Porphyria Brit M J 1 640 1958
290 Calvert R J and Rimington C Porphyria cutanea tarda in relapse a case report Brit M J 2 1131 1953
291 Discombe G and Treip C S Cutaneous manifestations of porphyria Brit M J 2 1134 1953
292 Berger H and Goldberg A Hereditary coproporphyria Brit M J 2 85 1955
293 Varadi S *Haematological aspects in a case of erythropoietic porphyria* Brit J Haemat 4 270 1958
294 Chaudhuri A Chaudhuri J N and Chaudhuri C C Congenital porphyria in siblings with a brief review of congenital and hereditary porphyrias Indian J Pediat 25 157 1958
295 Guimaraes N A Monteira A B Lisboa A and da Costa Pereira C G Congenital porphyria Hospital (Rio de Janeiro) 51 33 1957
296 Çam C Intoksikasyona bagli der porfirinleri Dirim 34 II 1959
297 Özen M A and Cetingil A I Personal communication

# Chapter 31

# Hereditary Spherocytosis

---

*James H. Jandl*

In hereditary spherocytosis a hemolytic process is associated with an intrinsic defect in the metabolism and structure of the red cell. The cardinal features of this disease are (1) spherocytosis, (2) increased rate of red cell turnover, (3) splenomegaly, (4) familial occurrence, and (5) almost invariable benefit from splenectomy. There are many synonyms for this hemolytic disease, the most common of which are congenital hemolytic anemia and congenital hemolytic jaundice.

*Incidence*

Although not restricted to any single race, hereditary spherocytosis is most frequent in people of European origin. Among those of Northern European descent it is the most prevalent of the hereditary hemolytic disorders. This disease affects the sexes equally and often is manifest in early infancy [*1, 2*].

*Clinical Description*

The major symptoms arise from anemic hypoxia. The severity of the anemia varies from time to time and from patient to patient. As the result of increased red cell destruction, a mild degree of acholuric jaundice is usually present, although it may be variable or intermittent and tends to be less pronounced in early childhood [*2*]. Gallstones of the pigment type are common, even in childhood [*3*], and presumably are precipitated from bile containing high concentrations of bilirubin. The spleen is usually palpable, but the liver is usually normal in size. Although less frequent than in sickle-cell anemia, chronic leg ulcers occasionally develop, particularly in adulthood. These are usually bilateral, just above the malleoli, and ordinarily disappear after splenectomy. A wide assortment of skeletal abnormalities has been described in patients with hereditary spherocytosis. Some of these are presumably caused by the expanding

morphologically from those appearing in acquired anemias manifesting spherocytosis such as hemolytic anemias caused by 'autoantibodies' or by certain oxidant drugs In hereditary spherocytosis the red cell mean corpuscular volume is usually in the normal or low normal range while the hemoglobin concentration (MCHC) tends to be above normal The reverse is usually true of the red cells in acquired hemolytic anemia with spherocytosis The red cell in hereditary spherocytosis is therefore deficient in surface area [7]

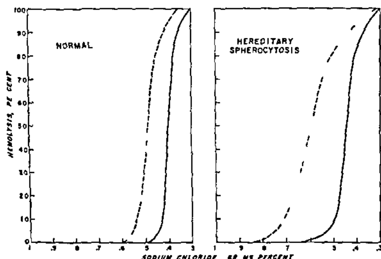



Fig 31 1 Comparison of osmotic fragility curves of normal red cells and of hereditary spherocytes These are summation curves depicting the proportion of red cells hemolyzed at various concentrations of sodium chloride they represent average values as determined by Emerson and his associates [13] As indicated by the continuous lines (freshly drawn) hereditary spherocytes are on the average slightly more susceptible to hypotonic lysis than normal red cells and a small portion of these cells is striking in this respect as indicated by a leftward 'tail' in the osmotic fragility curve After 24 hr of sterile incubation at 37 C (interrupted lines) hereditary spherocytes increase their thickness to diameter ratio more rapidly than do normal red cells as indicated by the greater leftward shift in the osmotic fragility curve

Spherocytes are identified by microscopic examination of peripheral blood smears or suspensions in which they appear as dark rounded cells lacking a central pale area and smaller in diameter and greater in thickness than normal red cells Spheroidicity may be measured in terms of osmotic fragility In the osmotic fragility test red cells are suspended in aqueous solutions containing various concentrations of sodium chloride Since there is almost no electrolyte exchange during the relatively short duration of the test osmotic equilibrium is achieved by the movement of water across the red cell membrane In hypotonic solution the red cell swells until it approaches a sphere Any further uptake of water abruptly

force of the hyperplastic erythroid bone marrow during the formative years

*Pathology*

As in other chronic hemolytic disorders there are compensatory normoblastic hyperplasia of the bone marrow and extension of the red marrow into the mid shafts of the long bones Occasionally extramedullary erythropoiesis may appear with formation of paravertebral masses The spleen as observed at operation is darkly congested with blood and usually weighs from 500 to 2 000 gm although a precise weight is often difficult to establish because of a continuing leakage of blood On section of the spleen the sinuses are often observed to be dilated and empty whereas the pulp cords are congested with red cells The significance of this finding is uncertain since recent studies [4] of ultrathin sections of the normal spleen indicate that the splenic sinuses and the splenic cords *are merely different functional phases of the same anatomic structure*

*Laboratory Findings*

The presenting laboratory findings are those common to all chronic hemolytic processes anemia an increased concentration of reticulocytes an increased concentration of indirect reacting (largely nonglucuronide) bilirubin in the serum and an increased rate of fecal urobilinogen excretion There is little or no hemoglobin in the plasma The hemoglobin which is released from destroyed red cells is catabolized to bilirubin at the site of destruction (so called extravascular hemolysis)

The severity of the manifestations of the disease depends upon the rate of red cell destruction which may range from slightly to greatly increased and the rate of compensatory red cell production by the marrow The erythropoietic response to anemia in this disease is unusually brisk Accordingly the anemia may be minimal despite moderately rapid rates of red cell destruction Patients having only slight anemia with disproportionately severe icterus and reticulocytosis are said to have a "compensated form of hemolytic anemia [5] On the other hand the equilibrium between the rates of red cell destruction and production may be dramatically upset by episodes of bone marrow failure ( aplastic crises ) during which erythroid hypoplasia and reticulocytopenia may ensue [6] Most commonly such episodes are precipitated by infections These infections may be otherwise mild but if highly communicable may cause an epidemic of aplastic crises in an afflicted family

*The Spherocyte*

The characteristic feature of this type of hereditary hemolytic anemia is the spherocyte (more accurately a cell more nearly spheroidal than the normal red cell) *The red cells in hereditary spherocytosis may differ*

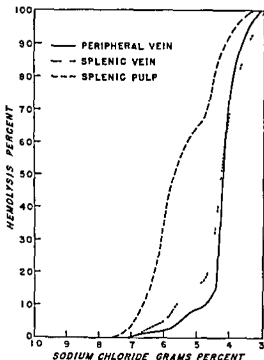



Fig 31-2 Influence of the spleen on the osmotic fragility of hereditary spherocytes The majority of the red cells were only slightly more spherical than normal However 10 per cent of the red cells in the peripheral blood (continuous line) represent an apparently separate population which distributes around a maximum increment of osmotic lysis at a sodium chloride concentration of between 0 55 and 0 60 gm per cent A similar but proportionately larger population of spherical cells was found simultaneously emerging from the splenic vein (dotted line) in the splenic pulp itself (interrupted line) the highly spherical population of red cells constituted a majority of those present Observations such as this indicate that hereditary spherocytes trapped in the spleen undergo further spherical transformation much as they do on incubation in vitro (Fig 31-1)

underlying the spherical shape and the abnormal susceptibility to injury by erythrostasis

## BIOCHEMICAL ABNORMALITIES OF HEREDITARY SPHEROCYTOSIS

### FUNCTION OF THE NORMAL RED CELL

Although the immature red cell is uniquely concerned with the synthesis of hemoglobin it is otherwise comparable in its metabolic activity to many other tissue cells When it matures to an adult red cell shortly after its release into the circulation its function becomes primarily that of transporting oxyhemoglobin This function requires the preservation

renders the cell membrane porous causing leakage of intracellular contents including hemoglobin Accordingly assuming that the cells have a normal internal osmolarity and providing the pH is kept constant the osmotic fragility test is a precise measure of how nearly spherical a cell is at the time of exposure to a hypotonic medium [8] Under conditions of this test the red cells including the newly formed reticulocytes [9] of patients with hereditary spherocytosis usually manifest an increase in osmotic fragility Thus spheroidicity is attained at higher concentrations of sodium chloride than is the case with normal red cells (Fig 31 1)

## MECHANISM OF RED CELL DESTRUCTION

### *Red Cell Survival*

Hereditary spherocytes have a diminished life span in the patient or in the normal subject when the spleen is present but their survival is almost normal in the splenectomized patient or normal subject [10] Normal red cells survive normally when transfused into patients with hereditary spherocytosis [11] Thus the red cell defect is intrinsic is nontransferrable and causes destruction of the cell only in the presence of the spleen

### *Role of the Spleen*

Although spherocytosis persists after splenectomy the excessive destruction of the spherocyte ceases The following observations have shown that the spleen is the actual and sole site of abnormal red cell destruction and does not simply exert an intermediate influence (1) The spleens of patients are characteristically engorged with blood and the bilirubin content of splenic vein blood considerably exceeds that of the peripheral blood [12] (2) Hereditary spherocytes labeled with $Cr^{51}$ and reinjected into patients or into normal subjects are sequestered in the spleen but not elsewhere [13 14] and prior to actual sequestration their circulation time through the spleen is abnormally slow [15]

Direct observations of splenic blood in hereditary spherocytosis have shown that the red cells in the splenic pulp are more nearly spherical than those in the peripheral circulation [16–19] (Fig 31 2) Spherocytes are selectively retained in the pulp of spleens as determined after transfusion *in vivo [16–20] and after splenic perfusion in vitro [18]* Indeed it has recently been shown [21] that spherocytes can be separated from normal red cells simply by passage through mechanical (Millipore) filters in vitro These observations support in all essential details the hypothesis of Ham and Castle [22 23] that hereditary spherocytes are trapped in the spleen by virtue of their increased thickness There they are subject to hemoconcentration [24] and erythrostasis and undergo metabolic changes to which they are peculiarly susceptible leading to their destruction In recent years efforts have been made to identify the biochemical lesion

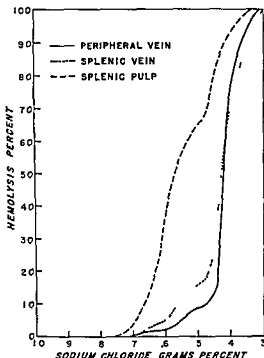



Fig 31 2 Influence of the spleen on the osmotic fragility of hereditary spherocytes The majority of the red cells were only slightly more spherical than normal However 10 per cent of the red cells in the peripheral blood (continuous line) represent an apparently separate population which distributes around a maximum increment of osmotic lysis at a sodium chloride concentration of between 0 55 and 0 60 gm per cent A similar but proportionately larger population of spherical cells was found simultaneously emerging from the splenic vein (dotted line) in the spleen pulp itself (interrupted line) the highly spherical population of red cells constituted a majority of those present Observations such as this indicate that hereditary spherocytes trapped in the spleen undergo further spherical transformation much as they do on incubation in vitro (Fig 31 1)

underlying the spherical shape and the abnormal susceptibility to injury by erythrostasis

## BIOCHEMICAL ABNORMALITIES OF HEREDITARY SPHEROCYTOSIS

### FUNCTION OF THE NORMAL RED CELL

Although the immature red cell is uniquely concerned with the synthesis of hemoglobin it is otherwise comparable in its metabolic activity to many other tissue cells When it matures to an adult red cell shortly after its release into the circulation its function becomes primarily that of transporting oxyhemoglobin This function requires the preservation

renders the cell membrane porous causing leakage of intracellular contents including hemoglobin Accordingly assuming that the cells have a normal internal osmolarity and providing the pH is kept constant the osmotic fragility test is a precise measure of how nearly spherical a cell is at the time of exposure to a hypotonic medium [8] Under conditions of this test the red cells including the newly formed reticulocytes [9] of patients with hereditary spherocytosis usually manifest an increase in osmotic fragility Thus spheroidicity is attained at higher concentrations of sodium chloride than is the case with normal red cells (Fig 31 1)

## MECHANISM OF RED CELL DESTRUCTION

### *Red Cell Survival*

Hereditary spherocytes have a diminished life span in the patient or in the normal subject when the spleen is present but their survival is almost normal in the splenectomized patient or normal subject [10] Normal red cells survive normally when transfused into patients with hereditary spherocytosis [11] Thus the red cell defect is intrinsic is nontransferrable and causes destruction of the cell only in the presence of the spleen

### *Role of the Spleen*

Although spherocytosis persists after splenectomy the excessive destruction of the spherocyte ceases The following observations have shown that the spleen is the actual and sole site of abnormal red cell destruction and does not simply exert an intermediate influence (1) The spleens of patients are characteristically engorged with blood and the bilirubin content of splenic vein blood considerably exceeds that of the peripheral blood [12] (2) Hereditary spherocytes labeled with $Cr^{51}$ and reinjected into patients or into normal subjects are sequestered in the spleen but not elsewhere [13 14] and prior to actual sequestration their circulation time through the spleen is abnormally slow [15]

Direct observations of splenic blood in hereditary spherocytosis have shown that the red cells in the splenic pulp are more nearly spherical than those in the peripheral circulation [16–19] (Fig 31 2) Spherocytes are selectively retained in the pulp of spleens as determined after transfusion *in vivo* [16–20] and after splenic perfusion *in vitro* [18] Indeed it has recently been shown [21] that spherocytes can be separated from normal red cells simply by passage through mechanical (Millipore) filters *in vitro* The observations support in all essential details the hypothesis of Ham and Castle [22 23] that hereditary spherocytes are trapped in the spleen by virtue of their increased thickness There they are subject to hemoconcentration [24] and erythrostasis and undergo metabolic changes to which they are peculiarly susceptible leading to their destruction In recent years efforts have been made to identify the biochemical lesion

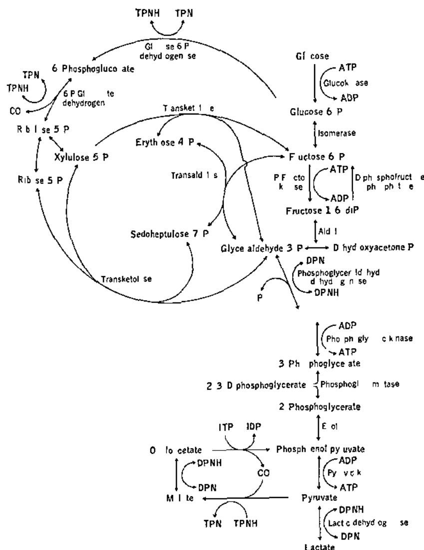



Fig 31 3 Metabolı m of glucose by red cells The main pathway in red cells is the anaerobic (Embden Meyerhof) pathway portrayed vertically in this scheme For every mole of glucose consumed in this chain of rea tions 2 moles of ATP and 2 moles of inorganic phosphate are ut lized in return 4 moles of ATP are generated and 2 moles of lactate are lost The cycl c pathway at the upper left rep es nts the hexo e mono phosphate sl unt (pentose pho phate pathway) whi h serves the important function of generating TPNH

of (1) hemoglobin molecules and (2) the physical integrity of the cell The preservation of hemoglobin depends to a large extent upon intra cellular reducing mechanisms and involves the formation of reduced triphosphopyridine nucleotide (TPNH) The metabolism of TPNH in the red cell is discussed in Chap 32 The metabolic activities necessary for the physical preservation of the cell are known to include the genera tion of high energy phosphate compounds It is in this respect that hereditary spherocytes appear to be defective

## PRESERVATION OF THE PHYSICAL STRUCTURE OF RED CELLS

The survival of the red cell in the circulation is critically dependent upon the preservation of smooth biconcave shape and upon maintenance of individual free suspensibility In its repetitive passage through the filtering systems of the body of which the spleen is the most exacting slight alterations in the thickness of the red cell or in the stickiness of its surface lead to sequestration and eventual destruction of the cell

The supporting structure of the red cell resides in the outer portion of the cell membrane and consists of a membrane [25] or a network [26] of lipoprotein The protein component a large complex fibrillar protein termed *elinin* [26] is insoluble and is probably metabolically inactive [27] The lipid components bound to this insoluble structural protein diffuse from red cells especially under nonphysiologic conditions [28] and exchange rapidly with plasma lipids [29] Some lipids may be actively synthesized by red cells [30] although this activity is very limited and probably occurs only in the youngest circulating cells [31] The actual processes which induce and maintain the biconcave structure of the red cell membrane are unknown

The volume and thickness of red cells depend upon ion transport across the cell membrane Since distribution of the anions chloride and bi carbonate appears to be passive maintenance of red cell volume depends largely on the distribution of cations The movement of cations across this membrane involves energy dependent processes in which potassium is actively concentrated in the cell and sodium is actively discharged Both ions are in a state of continuous flux [32–35] The energy for ion transport is derived from glucose metabolism [32–35]

## METABOLISM OF THE NORMAL RED CELL

### *Glycolysis*

Energy for the functions of the adult red cell is derived entirely from the metabolism of gluco e Unlike immature red cells including reticulocytes [36] the mature red cell lacks cytochromes and many of the components of the tricarboxylic acid cycle Since the red cell thus has little ability to utilize oxygen or to metabolize lactic acid glucose is mainly catabolized

fragility (Fig 31 1) [16 19 22] Swelling is accompanied by a decrease in electrolyte gradients permitting an influx of sodium and a lesser efflux of potassium [32 45] The net gain in electrolytes and water which results may reflect in part that sodium transport is more active than potassium transport [32–35] Indeed after 48 hr of incubation when the intracellular and extracellular concentrations of these cations have both approached equilibrium the red cells return to their original volume [45] However the mechanism of red cell swelling during incubation in vitro is still incompletely understood Swelling occurs to about the same extent when the cells are incubated in a potassium rich buffer as in a sodium rich buffer [46] Swelling is presumably due chiefly to oncotic pressure although an increase in intracellular osmolarity [47] may be contributary

During the first several hours red cell osmotic swelling is largely prevented by glucose unless glucose utilization is blocked by the addition of fluoride [32] Red cell swelling caused by prolonged storage is only partially inhibited by glucose and is attended by a depletion of cellular 2 3-diphosphoglycerate [42] and of ATP [48] and the appearance of free purines [49] These changes can be partially reversed by certain ribosides [50] particularly inosine [51] which are cleaved by purine nucleoside phosphorylase [42 52] possibly at the red cell membrane releasing ribose 1 phosphate [53] This ester through its conversion to ribose 5-phosphate presumably could be metabolized to triose phosphate by way of the hexose monophosphate shunt [50] (Fig 31 3) At least some purines may enter the erythrocyte there is recent evidence that adenine itself may also be utilized for ATP synthesis by intact rabbit red cells [54]

## METABOLISM OF HEREDITARY SPHEROCYTES

### *Abnormalities of Ion Transport*

The hemoglobin concentration in hereditary spherocytes frequently exceeds the upper limits of normal Although the exact explanation for this is lacking it is generally attributed to defective electrolyte regulation within the cell Potassium and water content are diminished [55] Although the sodium concentration is normal [45] the rate of sodium turnover is high [56 57]

An even more striking and consistent finding is the crucial observation that these cells are abnormally susceptible to erythrostasis When hereditary spherocytes are incubated at 37 C they become spheroidal even more rapidly than do normal red cells [17] (Fig 31 1) Indeed since these cells are usually partially spherical prior to incubation their exaggerated rate of increase of spheroidicity during stasis renders the measurement of osmotic fragility after incubation for 24 hr the most sensitive single test for detecting hereditary spherocytosis [17–19] This exaggerated increase

by the glycolytic (Embden Meyerhof) pathway (Fig. 21 3) and lactic acid accumulates. In addition to this pathway the mature red cell contains all the enzymatic components of an alternative oxidative pathway, the hexose monophosphate shunt [37] (pentose phosphate pathway, cf also Chap. 1). This pathway cycles glucose 6-phosphate through several intermediate steps, the first two of which are oxidation in which TPN serves as hydrogen acceptor. Reduced TPN is involved as a hydrogen donor in a number of processes important to the red cell including the reduction of methemoglobin and of oxidized glutathione. When the shunt is active for every three glucose molecules which take the pathway three molecules of carbon dioxide are released and two molecules of fructose 6-phosphate and one molecule of triose phosphate are formed [38]. Ordinarily this pathway is probably little utilized in red cells as indicated by the negligible oxidation of glucose to carbon dioxide in vitro. When substances such as methylene blue and other permeable and reversibly reducible dyes which may diffuse into the red cell are added this oxidative shunt is activated to full capacity and cellular respiration is increased dramatically [39, 40]. Such substances substitute for the otherwise deficient mechanism for carrying electrons directly from TPNH to oxygen. Possible utilization of the hexose monophosphate shunt in vivo has not been quantified but it is undoubtedly far less active than the anaerobic pathway.

Since the red cell is known to possess only a limited store of glycogen metabolic activity directly depends upon the availability of glucose in the surrounding medium. At physiologic plasma levels glucose can be rapidly transported across the cell membrane so as to maintain equal glucose levels in plasma water and cell water [41]. This transport does not require insulin [41].

*Phosphate Metabolism*

Phosphate for participation in red cell metabolism is obtained principally from the orthophosphate of plasma by direct incorporation into glyceraldehyde 3-phosphate, probably in the red cell membrane [42, 43]. The phosphorylated triose (1,3-diphosphoglycerate) acts as a phosphate donor in generating adenosine triphosphate (ATP), the more stable diester 2,3-diphosphoglycerate which has a uniquely high concentration in the red cell acts as a phosphate depot. In this respect the diphosphoglycerate occupy a strategic position in red cell energy metabolism comparable to that of creatine phosphate in muscle cells [44].

*Effects of Erythrostasis*

When normal red cells are incubated in vitro under sterile conditions, they undergo progressive structural and chemical changes. During the first 24 hr the cells swell and accordingly show an increase in osmotic

the cell membrane or is merely accentuated by the generally increased rate of intracellular phosphate turnover

*Effect of Splenectomy*

Although the exact nature of the biochemical defect in hereditary spherocytosis is not known it is apparent that the defect is not merely a consequence of a metabolic change induced by the spleen for the meta bolic abnormalities of the spherocytes are not influenced by splenectomy [60] The failure of splenectomy to affect the abnormal phosphate turn over of hereditary spherocytes does not disprove the possibility that this abnormality is in part acquired in the circulation The contribution of the spleen to the over all metabolism of the circulating red cells would be difficult to predict and may well be neutralized by the simultaneously opposing factors of (1) increased metabolic stress from splenic erythro sta is (2) the selection of a population of red cells having increased metabolic resistance by virtue of the filtration of spherocytes and (3) the accelerated release of young red cells from the marrow There remains the po sibility that a still more fundamental metabolic error exists in these cells and that phosphate metabolism is only secondarily affected

## GENETIC ASPECTS

The defect of hereditary spherocytosis is probably inherited as a Mendelian dominant characteristic This interpretation was first based on surveys which depended upon overt clinical and hematologic mani festations for identification of the defect [64–66] and has been confirmed in studies utilizing more precise laboratory criteria [1 67] If the disease is inherited as a Mendelian dominant trait one parent and half the siblings and offspring should be affected Although abnormal red cells are usually detectable in one parent of each propositus a number of instances have been reported in which the red cells of both parents were normal to existing tests [1 64 66 67] In two large series of patients there has emerged a significant deficit of affected siblings but no deficiency in the expected proportion of affected offspring [1 66]

There are several pos ible explanations for the deficit of affected rela tives (1) expression of the disease is highly variable [67] and may be conditioned by such environmental factors as the spleen there may well be some patients with red cell abnormalities of a mildness undetect able by the osmotic fragility method after incubation of the blood just as there are some with red cell abnormalities undetectable by this method without incubation (2) some cases may be a result of gene mutation a possibility which would explain the higher incidence in offspring than in siblings and parents (3) the deficiency in affected siblings may be partly a re ult of the unusually high rates of miscarriage and infant mortality

in spheroidicity during erythrostasis is not associated (at least initially) with an abnormal uptake of sodium and water from the surrounding plasma

Since the energy for ion transport depends upon glycolytic mechanisms attention has been focused upon the glucose metabolism of the hereditary spherocyte The abnormal degree of autohemolysis which ensues when hereditary spherocytes are incubated at 37°C for more than 48 hr can be largely prevented by the addition of glucose [46] Fluoride nullifies this effect of glucose [58] As with normal red cells the swelling of hereditary spherocytes exposed to the metabolic stress of erythrostasis is largely prevented by glucose or adenosine and related nucleosides [59]

*Abnormalities of Phosphate Metabolism*

The over all rates of glucose consumption [46] and of phosphate exchange [60] in hereditary spherocytes were found to be normal However the observations of Prankerd Altman and Young [60] revealed a consistent abnormality in the intracellular distribution of phosphate When hereditary spherocytes were incubated in plasma containing $P^{32}$ labeled orthophosphate there was a disproportionately small recovery of $P^{32}$ from the intracellular and stromal phosphate esters and a proportionately greater recovery of $P^{32}$ from the inorganic phosphate pool of the cell In the red cells of over half the patients studied these workers [60] also found that the addition of adenosine during incubation of hereditary spherocytes with $P^{32}$ increased toward normal the portion of $P^{32}$ which could be recovered from phosphate esters Since Prankerd et al interpreted the cellular concentrations of ATP ADP 2,3 diphosphoglycerate and inorganic phosphate to be normal they attributed the abnormal distribution of $P^{32}$ to a diminished rate of turnover of intracellular organic phosphate rather than to changes in the sizes of the respective phosphate pools [60] Inorganic and ester phosphate levels in the hereditary spherocytosis cell by more recent calculations [61] may be larger than those originally presented [60] Thus it appears that the movement of phosphate into the various intermediates deviates from normal but the exact nature of this abnormality is presently unclear

A specific enzymatic defect in hereditary spherocytes has not been described The finding that the uptake and retention of phosphate by hereditary spherocytes is impaired by a concentration of sodium fluoride ($10^{-3}$ *M*) which has little or no effect upon normal red cells [62] suggests the possibility that $Mg^{++}$ activated enzymes are involved One of these enzymes adenosine triphosphatase has been found to be normally active in hereditary spherocytes [63] thus abnormalities of both enolase [62] and a phosphokinase [63] have been proposed by exclusion It is equally possible that the sensitivity of hereditary spherocytes to fluoride poisoning is a function of the structure permeability or enzyme placement in

4 One hypothesis which can be proposed is as follows

*a* An abnormality in intermediary carbohydrate metabolism in hereditary spherocytes renders them peculiarly dependent upon the level of glucose and other metabolites in their environment Consequently when hereditary spherocytes are separated from a continuous supply of glucose containing plasma as in erythrostasis an abnormally rapid depletion of high energy phosphate compounds occurs ion transport breaks down and the cells increase in spheroidicity at an abnormally rapid rate This conditioning of the red cells by repetitious passage through the spleen over a period of approximately 10 days [*70*] creates a minor population of osmotically very fragile thickened red cells

*b* With the resulting increase in its thickness the hereditary spherocyte is unable to pass at a normal rate through the splenic sinuses and is thereby subjected to further erythrostasis and erythrocytic concentration which deprive it of metabolic substrate Being abnormally susceptible to such deprivation the cell becomes even more spheroidal is unable to escape from the spleen and eventually is destroyed by dissolution of the cell membrane

5 An alternative hypothesis is that structural organization alone may be responsible for the changes found in hereditary spherocytosis and that this abnormality of surface may account for the trapping tendency in the spleen

6 Hereditary spherocytosis therefore represents an inherited disease of the red cell The energy requirements of the red cell are relatively so low and its physical deformity of so little consequence in the general circulation that this metabolic defect would be harmless but for the exceptional capacity of the spleen to filter out abnormal cells and to subject them to stasis

## BIBLIOGRAPHY


1 Young L E Izzo M J and Platzer R F Hereditary spherocytosis I Clinical hematologic and genetic features in 8 cases with particular reference to the osmotic and mechanical fragility of incubated erythrocytes Blood 6 1073 1951

2 Dacie J V *The Haemolytic Anaemias* p 5 Grune & Stratton New York 1951

3 Gairdner D The association of gallstones with acholuric jaundice in children Arch Dis Childhood 14 109 1939

4 Weiss L A study of the structure of splenic sinuses in man and in the albino rat with the light microscope and the electron microscope J Biophys & Biochem Cytol 3 599 1957

5 Gänslen M Zipperlen F and Schüz I Die hämolytische Konstitution Deutsch Arch klin Med 146 1 1925

6 Owren P A Congenital hemolytic jaundice The pathogenesis of the hemolytic crisis Blood 3 231 1948

7 Crosby W H The pathogenesis of spherocytes and leptocytes (target cells) Blood 7 261 1952

It has not yet been possible to design experiments which will choose between these alternatives

A number of reports have described the association of hereditary spherocytosis with a variety of constitutional or skeletal defects including tower skull, polydactyly arched palate eye abnormalities and ovarian hypofunction [5 68 69] It is not certain, however that the reported incidence exceeds that of the general population There is apparently no genetic linkage between hereditary spherocytosis and such inherited constitutional features as sex blood group eye color or ear form [66]

There is no definite example of homozygous hereditary spherocytosis although it has been suspected in a family of 13 children all with hereditary spherocytosis 9 of whom manifested physical or mental retardation [69] Race [66] reported a mating of first cousins both of whom had hereditary spherocytosis Three of the children were affected one was normal and there were two miscarriages

An interesting observation which emerged from the metabolic studies of hereditary spherocytes was that at least one abnormality observed in hereditary spherocytosis has a familial pattern It was reported that the defect in intracellular partition of $P^{32}$ could be largely corrected by adenosine in cells from 10 of 18 patients investigated [60] In this respect all afflicted members of each family behaved uniformly, regardless of the severity of the anemia [60] The biochemical basis for this effect of adenosine is unknown but it cannot be ascribed simply to a deficit in purine nucleoside phosphorylase [60] This apparent genetic subdivision of patients with hereditary spherocytosis may reflect the existence of two (or more) basic disease entities manifesting in common an inherited spherocytosis

## SUMMARY

1 Hereditary spherocytosis is a congenital hemolytic anemia caused by an intrinsic defect in the metabolism of the red cell which is transmitted as a Mendelian dominant characteristic

2 The exact nature of this defect is unknown It is manifested by the formation in the bone marrow of red cells which are deficient in surface and which thereby approach a spherical rather than a normal discoidal form This deformity is associated with an abnormality in the metabolism of high energy phosphate compounds by the red cell Whether this metabolic abnormality is primary or simply secondary to an alteration of the structural organization of the cell membrane is as yet uncertain

3 Since unlike most body cells red cells lack intrinsic mechanisms for the direct utilization of oxygen and depend almost entirely on an aerobic glycolysis for the generation of energy there is rapid depletion of the energy sources necessary for ion transport and for maintenance of cell structure under erythrostatic conditions

31 Marks P A and Gellhorn A Lipid synthesis in human leukocytes and erythrocytes in vitro Fed Proc 18 281 1959
32 Maizels M Cation control in human erythrocytes J Physiol 108 247 1949
33 Raker J W Taylor I M Weller J M and Hastings A B Rate of potassium exchange of the human erythrocyte J Gen Physiol 33 691 1950
34 Harris E J and Maizels M The permeability of human erythrocytes to sodium J Physiol 113 506 1951
35 Solomon A K The permeability of the human erythrocyte to sodium and potassium J Gen Physiol 36 57 1952
36 Rapoport S and Hofmann E C G Untersuchungen uber den Atmungsstoffwechsel von Reticulocyten Biochem Ztschr 326 493 1955
37 Cohen S S In *Chemical Pathways of Metabolism* edited by D M Greenberg p 187 Academic Press Inc New York 1954
38 Wolfe S J A recently recognized pathway for glucose catabolism—pentose phosphate pathway A M A Arch Int Med 102 493 1958
39 Guzman Barron E S and Hoffman L A The catalytic effect of dyes on the oxygen consumption of living cells J Gen Physiol 13 483 1930
40 Brin M and Yonemoto R H Stimulation of the glucose oxidative pathway in human erythrocytes by methylene blue J Biol Chem 230 307 1958
41 Park C R Post R L Kalman C F Wright J H Jr Johnson I H and Morgan H E The transport of glucose and other sugars across cell membranes and the effect of insulin in Ciba Foundation Colloquia on Endocrinology vol IX *Internal Secretions of the Pancreas* p 240 Little Brown Boston 1956
42 Prankerd T A J and Altman K I A study of the metabolism of phosphorus in mammalian red cells Biochem J 58 622 1954
43 Bartlett G R Organization of red cell glycolytic enzymes cell coat phosphorus transfer Ann New York Acad Sc 75 110 1958
44 Prankerd T A J The metabolism of the human erythrocyte a review Brit J Haematol 1 131 1955
45 Selwyn J G and Dacie J V Autohemolysis and other changes resulting from the incubation in vitro of red cells from patients with congenital hemolytic anemia Blood 9 414 1954
46 Emerson C P Jr and Jandl J H Unpublished observations
47 Emerson C P Jr Unpublished observations
48 Rapoport S Dimensional osmotic and chemical changes of erythrocytes in stored blood I Blood preserved in sodium citrate neutral and acid-citrate glucose (ACD) mixtures J Clin Invest 26 591 1947
49 Jørgensen S Adenine nucleotides and oxypurines in stored donor blood Acta Pharmacol Toxicol 13 10 1957
50 Gabrio B W Hennessey M Thomasson J and Finch C A Erythrocyte preservation IV In vitro reversibility of the storage lesion J Biol Chem 215 357 1955
51 Gabrio B W Donohue D M Huennekens F M and Finch C A Erythrocyte preservation VII Acid-citrate-dextrose inosine (ACDI) as a preservative for blood during storage at 4 C J Clin Invest 35 657 1956
52 Sandberg A A Lee G R Cartwright G E and Wintrobe M M Purine nucleoside phosphorylase activity of blood I Erythrocytes J Clin Invest 34 1823 1955
53 Prankerd T A J Cleavage of adenosine by human red cells Brit J Haematol 1 406 1955
54 Lowy B A Ramot B and London I M Adenosine triphosphate metabolism in the rabbit erythrocyte in vivo and in vitro Ann New York Acad Sc 75 148 1958
55 Maizels M The anion and cation contents of normal and anaemic bloods Biochem J 30 821 1936
56 Harris E J and Prankerd T A J The rate of sodium extrusion from human erythrocytes J Physiol 121 470 1953
57 Bertles J F Sodium transport across the surface membrane of red blood cells in hereditary spherocytosis J Clin Invest 36 816 1957

8 Castle W B and Daland G A Susceptibility of erythrocytes to hypotonic hemolysis as a function of discoidal form Am J Physiol 120 371 1937
9 Paolino W Variations of the mean diameter in the ripening of the erythrocyte Acta med scandinav 136 141 1949
10 Schrumpf C A A *Role of the Spleen in Familial Spherocytosis* Proceedings of the Third International Congress International Society of Hematology 1950 p 94 Grune & Stratton New York 1951
11 Dacie J V and Mollison P L Survival of normal erythrocytes after transfusion to patients with familial haemolytic anaemia (acholuric jaundice) Lancet 1 550 1943
12 Gripwall E Zur Klinik und Pathologie des hereditären hämolytischen Ikterus Mit besonderer Berucksichtigung des Verhaltens der roten Blutkörperchen Acta med scandinav Suppl 96 1938
13 Jandl J H Greenberg M S Yonemoto R H and Castle W B Clinical determination of the sites of red cell sequestration in hemolytic anemias J Clin Invest 35 842 1956
14 Schloesser L L Korst D R Clatanoff D V and Schilling R F Radioactivity over the spleen and liver following the transfusion of chromium[51] labelled erythrocytes in hemolytic anemia J Clin Invest 36 1470 1957
15 Harris I M McAlister J M and Prankerd T A J The relationship of abnormal red cells to the normal spleen Clin Sc 16 223 1957
16 Emerson C P Jr Shen S C and Castle W B The osmotic fragility of the red cells of the peripheral and splenic blood in patients with congenital hemolytic jaundice transfused with normal red cells J Clin Invest 25 922 1946
17 Emerson C P Jr Shen S C Ham T H and Castle W B The mechanism of blood destruction in congenital hemolytic jaundice J Clin Invest 26 1180 1947
18 Young L E Platzer R F Ervin D M and Izzo M J Hereditary spherocytosis II Observations on the role of the spleen Blood 6 1099 1951
19 Emerson C P Jr Shen S C Ham T H Fleming E M and Castle W B Studies on the destruction of red blood cells IX Quantitative methods for determining the osmotic and mechanical fragility of red cells in the peripheral blood and splenic pulp the mechanism of increased hemolysis in hereditary spherocytosis (congenital hemolytic jaundice) as related to the functions of the spleen A M A Arch Int Med 97 38 1956
20 Weisman R Jr Hurley T H Harris J W and Ham T H Studies of the function of the spleen in the hemolysis of red cells in hereditary spherocytosis and sickle cell disorders J Lab & Clin Med 42 965 1953
21 Jandl J H Sequestration of reticulocytes and of abnormal red cells by filtration at low pressures J Clin Invest 37 905 1958
22 Ham T H and Castle W B Relation of increased hypotonic fragility and of erythrostasis to the mechanism of hemolysis in certain anemias Tr A Am Physicians 55 127 1940
23 Ham T H and Castle W B Studies on the destruction of red blood cells Relation of increased hypotonic fragility and of erythrostasis to the mechanism of hemolysis in certain anemias Proc Am Phil Soc 82 411 1940
24 Barcroft J and Poole L T The blood in the spleen pulp J Physiol 64 23 1927
25 Ponder E Present concepts of the structure of the mammalian red cell Blood 9 227 1954
26 Moskowitz M and Calvin M On the components and structure of the human red cell membrane Exper Cell Res 3 33 1952
27 Muir H M Neuberger A and Perrone J C Further isotopic studies on *haemoglobin formation* in the rat and rabbit Biochem J 52 87 195-
28 Lovelock J E The physical instability of human red blood cells Biochem J 60 692 1955
29 Gould R G Lipid metabolism and atherosclerosis Am J Med 11 209 1951
30 Altman K I The *in vitro* incorporation of $\alpha$ C acetate into the stroma of the erythrocyte Arch Biochem & Biophys 42 478 1953

# Chapter 32

# Drug induced Hemolytic Anemia (Primaquine Sensitivity)

*Ernest Beutler*

Over the years those charged with the care of the sick have often been dismayed to find that a drug that was innocuous to many patients produced catastrophic results in a few In 1953 it first became possible to study in detail one such drug sensitivity reaction the hemolytic effect of the 8 aminoquinoline antimalarial primaquine (Fig 32 1) These studies not only helped to clarify the mechanism of sensitivity to this drug but also led to the demonstration that a hereditary biochemical lesion of the erythrocyte was responsible for many other drug induced hemolytic anemias and for favism Because primaquine sensitivity is the prototype of this kind of hemolytic reaction only the history of 8 aminoquinoline hemolytic anemia will be reviewed That of related drug sensitivities is similar

The history of the 8 aminoquinoline compounds resembles that of many drugs the compound was hailed as causing no harm to the blood when it was first introduced in 1926 scarcely a year later severe even fatal hemolytic anemias were reported The first 8 aminoquinoline introduced to clinical medicine was plasmoquin (pamaquine) (Fig 32 1) which was used by Mühlens [*1*] to treat syphilitic patients who had been inoculated with malaria

Following Cordes [*2*] initial report of hemolytic anemia associated with the administration of this drug many similar reports appeared from all over the world [*3–35*] Typically the patient developed dark often black urine a few days after pamaquine therapy was begun Jaundice was noted and the red blood count and hemoglobin were found to be markedly diminished Usually the patient recovered but sometimes he succumbed to massive destruction of red blood cells

Attempts have been made to implicate immune mechanisms in the

58 Altman K I and Izzo M J quoted by Young L E Hereditary spherocytosis Am J Med 18 486 1955
59 Motulsky A G Gabrio B W Burkhardt J and Finch C A Erythrocyte carbohydrate metabolism in hereditary hemolytic anemias Am J Med 19 31 1955
60 Prankerd T A J Altman K I and Young L E Abnormalities of carbohydrate metabolism of red cells in hereditary spherocytosis J Clin Invest 34 1268 1955
61 Altman K I Personal communication
62 Tabechian H Altman K I and Young L E Inhibition of $P^{32}$-orthophosphate exchange by sodium fluoride in erythrocytes from patients with hereditary spherocytosis Proc Soc Exper Biol & Med 92 712 1956
63 Prankerd T A J Inborn errors of metabolism in red cells of congenital hemolytic anemias Am J Med 22 724 1957
64 Meulengracht E Über die Erblichkeitsverhaltnisse beim chronischen hereditaren hamolytischen Ikterus Deutsch Arch klin Med 136 33 1921
65 Campbell J M H and Warner E C Heredity in acholuric jaundice Quart J Med 19 333 1925–26
66 Race R R On the inheritance and linkage relations of acholuric jaundice Ann Eugenics 11 365 1942
67 Young L E Observations on inheritance and heterogeneity of chronic spherocytosis Tr A Am Phys 68 141 1955
68 Hansen K and Klein E Über Symptomatologie und Vererbung des hamolytischen Ikterus (Kugelzellenanamie) Deutsch Arch klin Med 176 567 1934
69 Bernard J Boiron M and Estager J Une grande famille hémolytique Treize cas de maladie de Minkowski Chauffard observés dans la même patrie Semaine hôp Paris 28 3741 1952
70 Griggs R C Weisman R Jr and Harris J W Alterations in osmotic and mechanical fragility related to in vivo erythrocyte aging and splenic sequestration in hereditary spherocytosis J Clin Invest 39 89 1960

compounds The clear cut demonstration by Dern et al [38] that sensitivity to the hemolytic anemia induced by these drugs was a property of the red blood cells made it possible to focus attention on the central problem the abnormality of the erythrocyte These studies demonstrated beyond doubt the important facts that hemolysis is limited to the older members of the red cell population [39] and that red cells sensitive to primaquine are sensitive also to a large number of other compounds [40]

Studies of the biochemistry of primaquine sensitive cells suggested that their sensitivity to hemolysis was related in some way to their glutathione (GSH) content [41] and the stability of their GSH [42] Examination of the pathways of GSH metabolism in drug sensitive red cells by Carson et al [43] disclosed the probable primary defect a deficiency of the enzyme glucose 6-phosphate dehydrogenase Subsequent observations established unequivocally that patients with favism have the same red cell defect as primaquine-sensitive subjects [44–56] Hemolytic anemia has been elicited in a subject with known sensitivity to fava beans by administration of primaquine [57] In addition it was found that red cells undergoing hemolysis following exposure of individuals to a wide variety of agents had the same biochemical defect as had been described in primaquine-sensitive red cells (Table 32 1)

TABLE 32 1 COMPOUNDS KNOWN TO HAVE INDUCED HEMOLYSIS OF PRIMAQUINE SENSITIVE RED CELLS

| | |
|---|---|
| Primaquine | Sulfacetamide [ 0] |
| Pamaquine [58] | Thiazolsulfone [40] |
| Pentaquine [59 60] | Antipyrine [60] |
| SN 3883 [59 60] | Probenecid [61] |
| CN 1110 [59 60] | Nitrofurantoin (Furadantin) [61] |
| SN 13274 [59 60] | Acetylsalicylic acid [6 ] |
| Sulfanilamide [40] | Furazolidone [6 ] |
| Acetanilid [ 0] | Sulfamethoxypyridazine (Kynex) [6 ] |
| Phenylhydrazine [40] | Salicylazosulfapyridine (Azulfadine) [6 ] |
| Sulfoxone [40] | Naphthalene [54 63 6 ] |
| Acetophenetidin (phenacetin) [40 41] | Para amino salicylic acid [6 ] |
| N₁ Acetylsulfanilamide [40] | Pyramidone [5 ] |

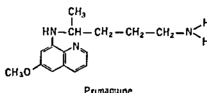
## HEMATOLOGY OF DRUG INDUCED HEMOLYTIC REACTIONS

### CLINICAL COURSE

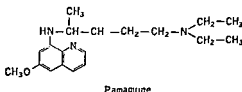
*Result of Drug Administration to a Sensitive Individual*

The clinical course of primaquine induced hemolysis has been studied under carefully controlled conditions by Dern et al [69] When a drug sensitive person is given 30 mg primaquine daily there is little or no

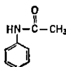
etiology of hemolytic anemia A nonspecific hemagglutinin was described in the blood of one patient [21], but it was demonstrated [33] subsequently that autoagglutinins could be found in the blood of patients receiving pamaquine who did not develop hemolytic anemia Skin testing was attempted [27] Fragility studies [11, 29, 33, 34] and Donath Landsteiner tests [19, 25] were carried out In addition, in vitro studies were made of hemolysis induced by pamaquine and related compounds or by the plasma

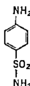


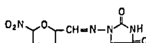

Primaquine




Pamaquine




Acetanilid Sulfanilamide Nitrofurantoin

Fig 32 1 Structural formulas of representative types of drugs which cause hemolytic anemia in primaquine sensitive subjects

of patients who had recently received pamaquine [29 36] None of these studies solved the problem of why some persons develop acute hemolytic anemia when given pamaquine and others do not

A few observations have been made which in retrospect were much more significant The appearance of Heinz bodies in the red cells of a patient with mild hemolytic anemia occurring during pamaquine therapy was noted in 1928 [8] Attention was called to the familial nature of pamaquine sensitivity and analogies were drawn between this disorder and favism [31] The difference in susceptibility of different races to hemolysis by pamaquine was recognized [18 22 25 27 29 33]

It was not until after the introduction of primaquine (Fig 32 1) a therapeutically more effective 8 aminoquinoline [37] that it became possible to study intensively the hemolytic anemia induced by the e

*Hemolytic Anemia in Nonsensitive Individuals*

Volunteer subjects have given an essentially all or none response to daily administration of 30 mg primaquine On the other hand large doses of those drugs which induce hemolysis in sensitive subjects may induce milder hemolysis in nonsensitive subjects The hemolytic effect of phenylhydrazine is well known In addition it has been demonstrated that acetanilid 3 6 gm daily or primaquine 120 mg daily will induce mild hemolysis in a normal person On the other hand some persons have taken 120 or even 240 mg primaquine daily without any evidence of hemolysis A combination of 90 mg primaquine and 3 6 gm acetanilid daily produced severe hemolysis in a normal subject [40] Para amino salicylic acid–induced hemolytic anemia has been described in a subject with nonsensitive red cells as judged by the GSH stability test and assay for glucose 6 phosphate dehydrogenase (see below) [75]

The hemolytic anemia caused by Fuadin has an immunologic basis [76] This drug induced hemolysis may arise by entirely different mechanisms in patients who are not primaquine sensitive

## DRUG SENSITIVITY AS AN INTRINSIC ABNORMALITY OF THE ERYTHROCYTE

Dern et al [38] transfused chromium 51 labeled red cells from a sensitive subject into nonsensitive recipients The survival of the transfused cells was normal until primaquine was administered to the nonsensitive recipient Rapid destruction of the $Cr^{51}$ labeled red cells then occurred The converse experiment was also carried out Chromium 51 labeled red cells from a normal individual were transfused into a known primaquine sensitive subject In contrast to the results obtained in the first experiment primaquine administration had no effect on the survival of the $Cr^{51}$ labeled cells from the normal donor although the primaquine-sensitive recipient underwent a typical acute hemolytic episode

## EFFECT OF CELL AGE ON HEMOLYSIS

A curious aspect of primaquine hemolytic anemia is that even if drug administration is continued during a hemolytic episode hemolysis will cease spontaneously When $Cr^{51}$ labeled red cells from a sensitive individual who had become tolerant to primaquine were transfused into a subject who had never previously received primaquine the labeled cells were unaffected when primaquine was given red cells from the sensitive subject were no longer susceptible to destruction When a primaquine-sensitive individual who was no longer destroying his own red cell although still receiving primaquine was given a transfusion of $Cr^{51}$

evidence of hemolysis during the first 2 or 3 days In contrast to immunologic sensitivity subsequent administration of drug does not shorten this latent period After 30 mg has been administered daily for 2 or 3 days the urine begins to turn dark In mild cases the patient may observe no other abnormality In more severe cases the patient complains of weakness and abdominal and back pain and develops icterus and black urine Heinz bodies appear in many of the red cells [70] the hemoglobin red blood cell and hematocrit values fall rapidly and the number of reticulocytes rises This ' acute hemolytic phase ends spontaneously in about 1 week even when drug administration is continued and the 'recovery phase begins The patient feels better the color of the urine becomes normal and the hemoglobin red cell and hematocrit values begin to rise The reticulocyte count remains high at first and then declines Throughout this time the Coombs test result is negative and red cell fragility remains unaltered The only morphologic changes in the blood are polychromasia and the appearance of Heinz bodies in the red cells Finally the peripheral blood picture returns to normal and the symptoms vanish even though administration of the drug is continued in the same dosage that initially caused hemolysis

Acetanilid and sulfanilamide [69] have also been given under controlled laboratory conditions to subjects known to be drug sensitive In addition there are many reports of hemolytic anemia induced accidentally by naphthalene [54 63–67 71] nitrofurantoin (Furadantin) [61 72] and fava beans [47 73] in persons who were demonstrated to be sensitive or who on clinical grounds may be presumed to have been sensitive Thus hemolytic anemia induced by acetanilid sulfanilamide Furadantin or naphthalene is similar to that induced by primaquine The self limited nature of the hemolytic anemia has not been demonstrated however with the latter two compounds Naphthalene induced hemolytic anemia may at times be considerably more explosive than that observed with primaquine administration

Fava bean–induced hemolysis may occur within hours of contact with fava beans and is often more severe than primaquine induced hemolysis Some factor other than the red cell defect of primaquine sensitivity is required for the production of fava bean–induced hemolysis in every case of favism in which the red cells have been studied the erythrocytic defect of primaquine sensitivity has been demonstrated but subjects known to have the latter defect have eaten fava beans with impunity [47 74]

Hemolysis induced by thiazolsulfone may vary greatly in intensity Careful studies of the mechanism of this variability suggest that it is due to differences in absorption or metabolism of the drug [69] In addition $Cr^{51}$ labeled primaquine sensitive red cells are not necessarily destroyed in vivo when fava beans are fed [74a]

## GSH METABOLISM AND ASSOCIATED PATHWAYS IN THE NORMAL ERYTHROCYTE

### OVER ALL METABOLISM OF THE ERYTHROCYTE

Several reviews of the metabolism of the mature mammalian erythrocyte have been published in the past decade [78 79] The metabolism of the erythrocyte which is exceedingly complex may be considered incomplete when compared with that of most other cells For example the mature red cell is unable to synthesize protein but does appear to

F g 3 ? Glu ose metal olism in the re l l loo l cell Al brev iated scl eme based on various sources [ 8 79] The site of the metabolic block in primaq uine-sensitive eryt] rocytes is indicated l y tl e double line between gluco ( pl ospl ate and 6-phosphogluconate

synthesize certain simpler compounds such as glutathione [80-82] nicotinamide mononucleotide and diphosphopyridine nucleotide [82a 82b] While it was at one time believed that fat ynthesis took place in erythrocytes [83 84] more recent studies indicate that lipid synthesis occurs only in reticulocytes and white cells [84a 84b] The red cell requires a source of energy for synthetic processes for the maintenance of ion concentration gradients and for the reduction of methemoglobin which is being formed continuously The chief source of energy of the red cell

labeled red cells from another primaquine-sensitive subject the transfused cells were destroyed rapidly the sensitive individual retained an undiminished capacity to destroy sensitive cells in his circulation Thus it was demonstrated that the refractory state which develops in primaquine-sensitive individuals is due not to altered metabolism of the drug after prolonged administration, but rather to an alteration in reactivity of the red cell population [69]

It was postulated that the red cell population might consist of a mixture of sensitive and nonsensitive red cells and that sensitivity might be a function of red cell age This hypothesis was confirmed by labeling the red cells of a sensitive subject with $Fe^{59}$ By bleeding the subject before administration of the radioisotope and by blocking reutilization with nonlabeled saccharated iron oxide administered intravenously it was possible to confine labeling to red cells of a relatively narrow age range Primaquine was administered when the tagged red cells were 8 to 21 days of age and again when the tagged cells were 63 to 76 days of age Destruction of labeled cells occurred only during the second course of drug administration even though the patient experienced an acute hemolytic episode during both courses of drug administration [39]

## STUDIES OF DRUG SENSITIVE ERYTHROCYTES BY ROUTINE HEMATOLOGIC TECHNIQUES

The abnormal response of the primaquine-sensitive subject is not another manifestation of a previously described red cell abnormality Coombs tests have been carried out [38 70] The Ham acid hemolysis test has been performed [70] Osmotic and mechanical fragility has been determined [70] Sickle-cell tests have been performed [58 70 77] and the electrophoretic mobility of the hemoglobin has been determined on paper at pH 8 6 [70] The percentages of alkali resistant hemoglobin [70] and methemoglobin [33 58 70] in the red cells have been measured These tests have failed to disclose any relationship between the defect of primaquine sensitivity and other intrinsic red cell abnormalities

Other studies have sought to determine how primaquine-sensitive red cells differ in vitro from normal red cells Lysis of primaquine- ensitive cells by primaquine pamaquine 6-methoxyquinoline quinoline and aniline was compared with lysis of normal red cells by the same compounds [70] The accelerating effects of primaquine on saponin hemolysis and of naphthalene on taurocholate hemolysis were measured [70] The effect of prolonged incubation with primaquine on osmotic mechanical and chemical fragility was observed [70] None of these studies showed any difference between primaquine sensitive and primaquine insensitive erythrocytes

*Reduction of Methemoglobin*

TPNH is utilized for the reduction of methemoglobin through the methemoglobin reductase system [97] in the presence of methylene blue The available evidence would suggest that this is probably not a physiologically important system [67] The concept that impairment of TPN reduction is not important in maintaining normal methemoglobin levels is supported by the finding that primaquine-sensitive cells in the presence of glucose and without methylene blue reduce methemoglobin at a normal rate [65] and by the fact that primaquine sensitive individuals do not develop greater degrees of methemoglobinemia than normal subjects [33, 58]

*Synthetic Processes*

TPNH may play an important role in a variety of reductive synthetic processes [98 99] While some of these probably do not occur in erythrocytes others may It may well be that here lies the most important role of TPNH in maintaining erythrocyte integrity

GSH METABOLISM

Reduced glutathione (GSH) is a tripeptide of glutamic acid cysteine and glycine It has one free sulfhydryl (—SH) group Recent reviews of the reactions of GSH may be consulted for more detailed description of its chemistry [100 101]

*Biosynthesis*

Isotopically labeled glycine is incorporated into the glutathione of mature mammalian erythrocytes [80 81] The finding that all three amino acids are labeled and that the distribution of radioactivity among them remains constant suggests that complete biosynthesis of GSH takes place rather than mere exchange or partial synthesis within the red cell [82]

*Oxidation*

GSH undergoes oxidation to the disulfide form (GSSG) under a variety of condition



$$GSH \rightleftharpoons GSSG + 2H$$

In pure solution GSH is relatively stable In the presence of traces of heme [102] metallic ion particularly copper [102] and selenite [103] it rapidly undergoes autoxidation to the disulfide form GSH may be oxidized by hemoglobin peroxide through the mediation of glutathione peroxidase [93] It appears likely that it can serve as a hydrogen donor

comes from the breakdown of glucose but in vitro inosine may be utilized as well (Fig 32 2)

*Anaerobic Metabolism*

Under physiologic conditions glucose metabolism in the red cell is primarily anaerobic and yields lactic acid The derived energy is stored in the form of high energy phosphate bonds by phosphorylation of adenosine diphosphate (ADP) to form adenosine triphosphate (ATP) During glycolysis 1 mole of diphosphopyridine nucleotide (DPN) is reduced in the oxidation of glyceraldehyde 3 phosphate to 1 3 diphosphoglyceric acid and 1 mole of reduced DPN is oxidized in the reduction of pyruvic to lactic acid

*Oxidation of Glucose—the Hexose Monophosphate (HMP) Pathway*

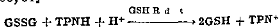
An alternate pathway of glucose metabolism is also available This is the hexose monophosphate shunt (see Chaps 3, 4 and 31) in which glucose undergoes oxidative catabolism Under normal conditions the HMP pathway accounts for only a small proportion of the glucose utilized by the red blood cell Methylene blue and certain other dyes readily activate this route of metabolism by linking it to molecular oxygen and to methemoglobin *[85–87]* Glucose is phosphorylated at the 6 position and then undergoes oxidation to 6 phosphogluconic acid through the action of glucose 6 phosphate dehydrogenase In this step 1 mole of triphosphopyridine nucleotide (TPN) is reduced Phosphogluconic acid then undergoes oxidation presumably forming 6 phospho 3 keto gluconate Again 1 mole of TPN is reduced The normal fate of the ribose 5 phosphate formed is less well understood Various cleavages and condensations may take place *[88 89]*

## PATHWAYS OF OXIDATION OF TPNH IN THE ERYTHROCYTE

Several alternate routes of oxidation of reduced TPN (TPNH) are available in the red cell Presumably these reflect some of the physiologic roles of TPNH and of the TPNH generating system in the red cell

*Reduction of Oxidized Glutathione (GSSG)*

TPNH is oxidized in the course of enzymatic reduction of oxidized glutathione *[90, 91]*



$$GSSG + TPNH + H^{+} \xrightarrow{\text{GSH R d t}} 2GSH + TPN^{+}$$

It has been suggested that a normal route of electron transfer from TPNH may involve the GSSG ⇌ GSH reaction *[92]* This might occur through oxidation of GSH by peroxide compounds *[93 94]* GSH is readily converted to GSSG in vivo red cell GSH di appears relatively rapidly when red cells are exposed to oxygen in the absence of glucose *[95, 96]*

vent the accumulation of peroxide hemoglobin complexes and thereby reduce choleglobin and methemoglobin formation in intact red cells which have been poisoned with azide [94] Yet it is not established that these are physiologic roles

Red cells poisoned with sulfhydryl reagents such as p chloromer curibenzoate hemolyze rapidly in vitro [118 119] Such experiments yield no reliable information regarding the need for GSH in the cell since these agents are nonselective in their inactivation of —SH groups It is also known that as the GSH of oxygenated shed blood decreases spontaneous hemolysis is accelerated [95] Since other changes also un doubtedly take place when blood is oxygenated in the ab ence of glucose the falling GSH and spontaneous hemolysis cannot be considered as having unequivocally a cause and effect relationship

GSH serves as coenzyme for glyoxalase known to be present in red cells [120] but no function is known for glyoxalase in the erythrocyte

It may be as suggested by Barron [121] for other tissues that GSH helps to maintain —SH groups in the reduced state It is of interest that glucose 6-phosphate dehydrogenase [122] and GSH reductase [118 115] both of which serve to keep GSH in the reduced state are inhibited by sulfhydryl reagents Unlikely though it seems it may be that GSH plays no obligatory role in the economy of the red cells Further work utilizing new approaches will be nece sary to elucidate the physiologic role of this compound in the red cell

## BIOCHEMISTRY OF DRUG SENSITIVE ERYTHROCYTES

### ABNORMALITIES FOUND INDEPENDENTLY OF DRUG ADMINISTRATION

#### *Glucose 6 Phosphate Dehydrogenase Activity Deficiency*

Dilute hemolyzates from drug sensitive erythrocytes are unable to promote reduction of GSSG normally when glucose 6-phosphate is the substrate Normal GSSG reduction takes place when TPNH or 6-phos phogluconate is supplied This is the basic observation in defining at the enzymatic level the difference between drug sensitive and nonsensitive erythrocytes [43] It indicates that the red cells of sensitive subjects are deficient in glucose 6-phosphate dehydrogenase activity This has been confirmed by many investigators using both the GSH linked assay pro cedure [50] and an assay method that measures directly the reduction of TPN by hemolyzates supplied with gluco e 6-pho phate as substrate [46 51 54 55 65] Mixtures of various proportions of normal and sensi tive hemolyzates do not reveal any enzyme inhibition [122a] The enzyme deficiency seems to be particularly severe in older red cells [123 123a] By using the GSSG linked assay system it has been shown that incubation with a nondialyzable stromal factor pre ent in both sen itive and non

for the reduction of methemoglobin [93, 94, 104] while undergoing oxidation to GSSG It is apparently oxidized rapidly when red cells are oxygenated [95, 96] In some tissues GSH may be oxidized by dehydroascorbic acid [105]

*Reduction*

GSSG may be reduced to GSH nonenzymatically by a variety of agents, including zinc [106] sodium amalgam [107] cysteine [108] and hydrogen sulfide [109] and may also be readily reduced electrolytically [110] A glutathione reductase [90] which mediates the reduction of the disulfide form with TPNH as the hydrogen donor, has been found in several rat tissues [90 111] and a number of plant tissues [90 112] DPN is ineffective as coenzyme [90 113]

Human red cells also contain a substance which catalyzes the reduction of GSSG with TPNH serving as a hydrogen donor [91 114] Reduced DPN will also serve as a hydrogen donor [91 114] in the case of red cell GSH reductase although somewhat less efficiently The relative efficiency of DPNH and TPNH as hydrogen donors has not been found to change in over one thousand fold purification with respect to protein concentration of the red cell enzymes [115] The degree of specificity of red cell ' glutathione reductase has not been studied It is not identical with methemoglobin reductase purified preparations of the latter do not promote TPNH oxidation in the presence of GSSG [115], GSSG reductase can be inhibited with p chloromercuribenzoate without inhibiting methemoglobin reductase [115] It is entirely possible that ' glutathione reductase represents the combined activity of several enzymes, and that some of these may be involved also in methemoglobin reduction This problem deserves further study

*Catabolism*

Aside from the interconversion of GSH and GSSG other pathways for the metabolism of red cell glutathione apparently exist at least in abnormal circumstances Thus when red cells are incubated with acetyl phenylhydrazine in the absence of glucose there is a decrease not only in GSH but also (after an initial increase) in GSSG [116 117] Since the analytical technique used in these determinations also measures cystine or cysteine it seems unlikely that some method of breakdown other than simple hydrolysis of the peptide bonds is involved It has also been suggested that GSSG is destroyed in ordinary hemolyzates [94]

*Function in the Economy of the Cell*

The function of GSH in the red cell must be regarded as unknown As has been pointed out GSH can serve as a hydrogen donor in the reduction of methemoglobin in hemolyzates [93 94 104] It also can pre

vent the accumulation of peroxide hemoglobin complexes and thereby reduce choleglobin and methemoglobin formation in intact red cells which have been poisoned with azide [94] Yet it is not established that these are physiologic roles

Red cells poisoned with sulfhydryl reagents such as p chloromercuribenzoate hemolyze rapidly in vitro [118 119] Such experiments yield no reliable information regarding the need for GSH in the cell since these agents are nonselective in their inactivation of —SH groups It is also known that as the GSH of oxygenated shed blood decreases spontaneous hemolysis is accelerated [95] Since other changes also undoubtedly take place when blood is oxygenated in the absence of glucose the falling GSH and spontaneous hemolysis cannot be considered as having unequivocally a cause and-effect relationship

GSH serves as coenzyme for glyoxalase known to be present in red cells [120] but no function is known for glyoxalase in the erythrocyte

It may be as suggested by Barron [121] for other tissues that GSH helps to maintain —SH groups in the reduced state It is of interest that glucose 6-phosphate dehydrogenase [122] and GSH reductase [113 115] both of which serve to keep GSH in the reduced state are inhibited by sulfhydryl reagents Unlikely though it seems it may be that GSH plays no obligatory role in the economy of the red cells Further work utilizing new approaches will be necessary to elucidate the physiologic role of this compound in the red cell

## BIOCHEMISTRY OF DRUG SENSITIVE ERYTHROCYTES

### ABNORMALITIES FOUND INDEPENDENTLY OF DRUG ADMINISTRATION

*Glucose 6 Phosphate Dehydrogenase Activity Deficiency*

Dilute hemolyzates from drug sensitive erythrocytes are unable to promote reduction of GSSG normally when glucose 6 phosphate is the substrate Normal GSSG reduction takes place when TPNH or 6-phosphogluconate is supplied This is the basic observation in defining at the enzymatic level the difference between drug sensitive and nonsensitive erythrocytes [43] It indicates that the red cells of sensitive subjects are deficient in glucose 6-phosphate dehydrogenase activity This has been confirmed by many investigators using both the GSH linked assay procedure [50] and an assay method that measures directly the reduction of TPN by hemolyzates supplied with glucose 6-phosphate as substrate [46 51 54 55 65] Mixtures of various proportions of normal and sensitive hemolyzates do not reveal any enzyme inhibition [122a] The enzyme deficiency seems to be particularly severe in older red cells [123 123a] By using the GSSG linked assay system it has been shown that incubation with a nondialyzable stromal factor present in both sensitive and non

sensitive cells results in loss of enzymatic activity from sensitive cells from at least some subjects but not from nonsensitive cells [50 124] A stromal factor, therefore appears to inactivate the enzyme in the drug sensitive cells but not in the nonsensitive cells The pH optimum of crude enzyme from normal and sensitive cells has been reported to differ [122a] However this observation has not been confirmed when more highly purified enzyme has been studied [125] Thus the apparent deficiency of the enzyme may in fact be due to an abnormal enzyme molecule which has lower activity and is especially susceptible to damage [122a 126], but much further work needs to be done to determine whether this is the case

There is considerable variability in the severity of enzyme deficiency observed in various subjects In addition recent studies have shown that there is a marked difference between the erythrocyte glucose 6 phosphate dehydrogenase levels of Negro and Caucasian subjects with this enzymatic deficiency The defect is much more severe in affected Caucasian subjects [126a]

Enzyme assays have also been carried out on the leukocytes platelets and liver tissues of sensitive subjects The leukocyte glucose 6-phosphate dehydrogenase activity of Negro subjects with red cell glucose 6-phosphate dehydrogenase deficiency is only slightly reduced and the statistical significance of the reduction is border line [126a] In the case of Caucasian subjects however a well marked reduction of glucose 6 phosphate dehydrogenase activity was observed in the leukocytes of affected individuals [126b] Reduced platelet enzyme levels have been found in sensitive Caucasian subjects [126b] A border line decrease in liver enzyme activity in affected subjects has been reported [126c]

*Effects of Glucose 6 Phosphate Dehydrogenase Deficiency*

**GSH Deficiency** Red cells from drug sensitive individuals are deficient in GSH and also in total glutathione (GSH + GSSG) [127 128] The GSH deficiency was demonstrated originally in primaquine sensitive subjects both by GSH assay with a nitroprusside method of relatively low specificity and by the use of the more specific alloxan 305 technique [41] Because almost all the nonprotein sulfhydryl of the red cell is GSH [129] the results obtained by means of these two methods were almost identical both yielding low values in drug sensitive and normal values in nonsensitive individuals The deficiency of red cell GSH in drug sensitive individuals is fairly consistent especially if the determinations on several days are averaged [41] There is some overlap of individual values [42] Low GSH values have also been observed in erythrocytes of subjects with hemolytic anemia due to fava beans or drugs other than primaquine [44 45 53 55] It has been claimed that the GSSG as well as the GSH of sensitive erythrocytes is decreased [128] This finding

has been disputed because of the relatively nonspecific assay method for GSSG which was employed [127]

**GSH Instability** There is rapid destruction of GSH [116] when red cells from a sensitive or non sensitive individual are suspended in a saline-phosphate buffer and incubated with acetylphenylhydrazine Concurrently there is an increase in the red cell GSSG level Subsequently the GSSG falls slightly, so that a significant decline in total glutathione takes place during the course of the experiment [117] If glucose or inosine

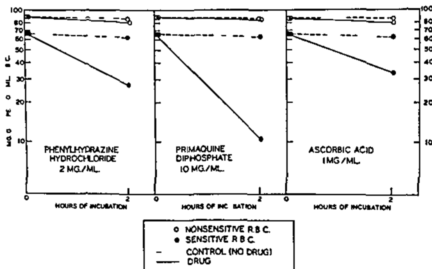



Fig 32-3 The effect of incubation at 37 C with phenylhydrazine primaquine and ascorbic acid on the GSH levels of sensitive and nonsensitive red cells [130]

is added to the system the nonsensitive red cells protect their GSH completely but protection of GSH in sensitive cells is incomplete [116 130] The same effect can be demonstrated also with other reducing agents e g primaquine phenylhydrazine ascorbic acid (Fig 32 3) [42 130] Furadantin [61] $\alpha$ and $\beta$ naphthoquinone [63] and certain Vitamin K derivatives [63 67] Substrates other than glucose and inosine including ribose lactate pyruvate malate and fumarate have little or no protective properties [116] The addition of methylene blue does not enhance the effectiveness of glucose in protecting red cell GSH [116]

The mechanism by which acetylphenylhydrazine destroys GSH in red cells in vitro has been investigated in some detail Oxygenation of the red cells is required for the destruction of GSH Carbon dioxide and CO are extremely effective in protecting the GSH of sensitive cells from destruction [116] (Fig 32-4) Acetylphenylhydrazine modifies oxyhemoglobin in such a way that it rapidly oxidizes GSH [116] The nature

of the intermediate compound has not been determined but it may be that a hemoglobin peroxide compound is formed and that GSH destruction is mediated by GSH peroxidase [94] It appears, therefore, that acetylphenylhydrazine induces a change in hemoglobin which causes it to oxidize rapidly the GSH of red cells Normal red cells have the capacity to reduce TPN by oxidizing glucose and to reduce the GSSG to GSH Normal cells deprived of glucose and cells lacking glucose 6 phosphate

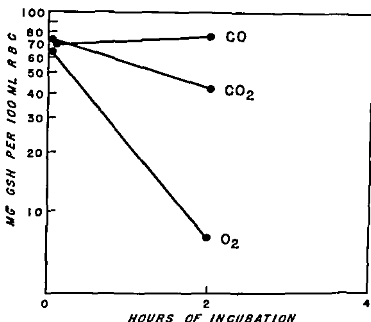



Fig 32-4 The effect of oxygen carbon dioxide and carbon monoxide on the GSH content of drug-sensitive red cells incubated with acetylphenylhydrazine Carbon monoxide and carbon dioxide exerted a pronounced protective effect (*From E Beutler [42] by permission of the Journal of Laboratory and Clinical Medicine*)

dehydrogenase do not reduce enough TPN to maintain GSH in the reduced state Apparently GSSG is destroyed as it accumulates within the red cell

An additional complication is that certain hemolytic compounds inhibit purified glucose 6-phosphate dehydrogenase [131] It remains to be seen what role this may play in bringing about GSH instability in intact red blood cells

**$CO_2$ Production and $O_2$ Consumption** Since the hexose monophosphate shunt is the only known pathway of oxygen consumption and $CO_2$ production in the red cell [86] an enzyme deficiency along this route would be expected to decrease $O_2$ consumption and $CO_2$ production This is indeed *the case when $O_2$ consumption and $CO_2$ production of* drug sensitive red cells incubated in the presence of methylene blue are

compared with those of normal cells [*123*] As expected the amount of $CO_2$ produced from the 1 position of labeled glucose was less in drug sensitive than in control cells [*123*] Furthermore comparison of the utilization of the 1 carbon and the 6-carbon of labeled glucose administered intravenously to drug sensitive and normal subjects has revealed marked retardation of utilization of the 1 carbon by drug sensitive subjects [*132*] This finding would imply that glucose 6-phosphate dehydrogenase deficiency is not limited to the red cells of sensitive subjects (see below)

**Methemoglobin Reduction** Reduction of methemoglobin in the presence of methylene blue a TPNH linked process is diminished in drug sensitive cells [*65*]

**TPN/TPNH Ratio** The ratio of TPN to TPNH is increased in sensitive cells [*133–135*] as might be predicted with a defect in the TPN reducing system A slight increase in the DPN/DPNH ratio has also been described [*135*]

**Compensatory Mechanisms** A number of other enzymatic changes have also been observed in primaquine sensitive red cells independently of drug administration Increased activity of red cell GSH reductase [*92*] aldolase [*55 134 136*] glyceraldehyde phosphate dehydrogenase [*55*] and lactic dehydrogenase [*55*] has been reported Activity of the last three enzymes has also been reported to be normal [*123 134 136a*] Some of these increased activities may represent compensatory changes which enable the red cell to live out a normal life span when not challenged by a hemolytic drug [*136*]

OTHER BIOCHEMICAL MEASUREMENTS IN SENSITIVE RED CELLS

The activity of phosphogluconic dehydrogenase [*54*] phosphohexose isomerase [*54*] purine nucleoside phosphorylase [*54*] triosephosphate isomerase [*134*] 3 phosphoglycerate kinase [*134*] pyruvate kinase [*134*] isocitric dehydrogenase [*136a*] and malic dehydrogenase [*136*] has also been measured in drug sensitive red cells and has been normal Enolase activity was normal in one subject but decreased in another [*134*] Chromatography of the amino acid residues of hydrolyzed hemoglobin from sensitive red cells demonstrated no abnormality [*48*] It has been claimed that a decrease in ADP and AMP as measured by enzymatic hydrolysis occurs in the whole blood of affected subjects [*134*] Using the more exact chromatographic technique of Bartlett [*137*] the author and coworker [*115*] have been unable to confirm this observation (see below) It has also been claimed that intracellular $K^+$ of affected cells is significantly diminished [*134*] No abnormality has been detected in stromal lipid fractions [*134*]

Chromatography of phosphorylated intermediary metabolites of the red cells of a drug sensitive individual has been carried out on Dowex 1

TABLE 32 2 RED CELL PHOSPHORYLATED INTERMEDIATES AS SEPARATED CHROMATOGRAPHICALLY ON A DOWEX 1 COLUMN

| *Subject* | *Cl cose 6-PO* | *Fructose 6-PO*$_4$ | *Hexose diphosphate* | *Adenosine mono-PO (AMP)* | *Adenosine di PO (ADP)* | *Adenosine tri PO*$_4$ *(ATP)* | *Dipho pho glycerate* |
|---|---|---|---|---|---|---|---|
| Normal 5 ubjects (mean ± 1 s d μM/100 ml) | 14 6 ± 6 6 | 8 7 ± 4 2 | 44 5 ± 12 3 | 19 1 ± 18 3 | 24 7 ± 15 9 | 50 0 ± 6 5 | 354 ± 80 |
| Drug ensitive 1 ubject μM/100 ml | 44 0 | 10 0 | 33 5 | 25 9 | 40 4 | 55 7 | 391 |

resin [115] employing the techniques developed by Bartlett [137] The results (Table 32 2) reveal an increase in hexose monophosphate and diphosphate which may readily be explained on the basis of a metabolic block in the direct oxidation of hexose monophosphate No other abnormalities were observed

## CHANGES OCCURRING IN ERYTHROCYTES DURING DRUG ADMINISTRATION

### *Glucose 6 Phosphate Dehydrogenase*

Only the older members of a red cell population are destroyed when primaquine is administered to a sensitive subject [39] Studies based on age fractionated red cells indicate that higher levels of glucose 6-phosphate dehydrogenase are present in young cells than in old cells [138–140] and that this disparity exists also when red cells from primaquine sensitive subjects are examined [123 123a 126a] Since a deficiency of glucose 6-phosphate dehydrogenase appears to be the basic defect of primaquine sensitive cells it would be most attractive to suppose that the higher levels of this enzyme in younger cells protect them against destruction and that the lower levels found in older members of the red cell population render them particularly sensitive to destruction

Dawson et al [65] have reported near normal glucose 6 phosphate dehydrogenase levels in subjects with naphthalene-induced hemolytic anemia and Sansone et al [141] have found normal levels and Grignani and Brunetti [141a] near normal levels in subjects with favic crises On the other hand while measurements of glucose 6-phosphate dehydrogenase activity during the administration of primaquine to sensitive subjects show a slight rise concurrently with the reticulocytosis which follows hemolysis enzyme activity soon returns to predrug administration levels according to Flanagan et al [127] This happens even though the subject is no longer sensitive to the hemolytic action of primaquine The latter observation is based on the GSH linked assay system and would seem to preclude the attractive explanation that the younger red cells of a drug sensitive individual owe their resistance to hemolysis to higher glucose 6-phosphate dehydrogenase activity and is difficult to reconcile with the findings on in vitro age-separated cells [123 123a 126a 138–140] The discrepancy in these results may be the result of the different assay methods

### *GSH*

The administration of primaquine to a drug-sensitive subject results in a rapid fall in the average GSH level of the red cell This fall precedes the major portion of red cell destruction There is then a return of average red cell GSH to the same or slightly higher level than prior to drug ex

hibition [127] Similar observations have been made in subjects with favism [49 53, 55, 56] and sulfonamide induced [49] hemolytic anemia It has been suggested that initially GSH in the older red cells is destroyed and that GSH depleted cells are then removed from the circulation [116]

The fate of GSH in sensitive red cells challenged in vivo by drug requires further study No increase in red cell GSSG has been observed concurrently with GSH destruction in vivo even though many observations have been made at frequent intervals This has been interpreted as indicating that the route of GSH destruction in vivo does not involve conversion to GSSG [127] This seems unwarranted, however, since it is entirely possible that the steady state condition of the red cell does not permit GSSG accumulation and that rapid degradation of GSSG takes place as it is formed It has been reported that the results of the GSH stability test (see below) are not affected by primaquine administration [127] However transient glutathione instability in red cells of sensitive subjects has been reported following naphthalene [65] and fava bean-induced hemolysis [49 55 141]

*Methemoglobin Formation*

The formation of methemoglobin in sensitive subjects given drug is not greater than that observed in nonsensitive subjects [33 58] and may even be less [60]

*Mechanism of Red Cell Destruction*

While much has been learned of the biochemical abnormalities of drug sensitive red cells and of the changes which occur during drug administration comparatively little is known of how these changes lead to red cell destruction when a potentially hemolytic drug is administered

Hemolytic drugs do not produce preferential lysis of drug sensitive red cells in vitro [70] Hence they must modify the red cell in such a way that it is selected for destruction presumably by the reticuloendothelial system While normally the spleen may play a predominant role in the destruction of red cells it has been found that even a splenectomized subject rapidly destroyed drug sensitive red cells transfused into him when drug was administered [38]

Heinz bodies which appear in the red cells at the onset of the hemolytic episode and which disappear as hemolysis progresses may represent a microscopically visible manifestation of the biochemical insult sustained by sensitive cells when drug is administered [70] The production of Heinz bodies can be reproduced in vitro it is enhanced by sulfhydryl reagents such as arsenite and iodoacetate Oxygen is required for their formation [142] Since they are believed to consist of particles of denatured protein [143] it is possible that the insult is one of protein

denaturation occurring under oxidative conditions and that sulfhydryl compounds play a defensive role

Red cell GSH is low in sensitive individuals and falls sharply under conditions of both in vivo [*127*] and in vitro [*42*] exposure to a drug For this reason this compound has received much attention in attempts at outlining the course of events leading to the destruction of drug sensitive red cells The suspicion that GSH is involved in this reaction is supported by the disappearance of GSH from cells just prior to their destruction [*127*] and by the fact that sulfhydryl reagents promote Heinz body formation in vitro [*142*] Nonetheless sulfhydryl reagents inhibit many enzymatic reactions within red blood cells and it is entirely possible that other more important but less easily measured changes which take place concurrently with GSH destruction may be the primary events leading to red cell destruction Caution must be taken not to interpret the easily measured GSH changes as being necessarily important in determining destruction of the red cell As pointed out in an earlier section the role of GSH in the red cell must be regarded as unknown

## METHODS OF DETECTION OF DRUG SENSITIVE ERYTHROCYTES

The in vivo challenge of erythrocytes with a drug is obviously an impractical means of studying the incidence or genetics of drug sensitivity For such studies it is necessary to depend on in vitro means for detection of drug sensitive persons Several methods for distinguishing apparently normal red cells of drug sensitive subjects from those of normal persons are now available

### HEINZ BODIES

When drug sensitive erythrocytes are incubated with a variety of reducing agents such as acetylphenylhydrazine phenylhydrazine primaquine or ascorbic acid a different pattern of Heinz body formation is observed than occurs when normal cells are incubated under identical conditions [*142*] This forms the basis of a method by which fairly reliable differentiation of sensitive from non sensitive subjects can be made [*55 61 67 141 142*] The chief advantage of this technique is the simplicity with which the test can be performed on large numbers of blood samples It is also the only method by which positive results are consistently obtained even when the patient is undergoing a hemolytic crisis [*127 141*] Disadvantages include the sensitivity of the test to changes in oxygen tension and to change in the hematocrit [*142*] It is not surprising therefore that false-positive results have been reported on subjects with thalassemia [*141*] Less easily explained are false-positive results observed in nonanemic subjects [*142*]

**Procedure** [*142*] A buffered acetylphenylhydrazine solution is prepared by dissolving 100 mg acetylphenylhydrazine in 100 ml of a M/15 phosphate buffer pH 7 6 containing 200 mg glucose per 100 ml Whole gently agitated venous blood 0 1 ml is added with a 'blow-out' pipet to 2 ml of the acetylphenylhydrazine solution in a test tube with an inner diameter of 12 mm The suspension is mixed immediately and aerated two or three times by drawing approximately 0 1 ml of the suspension into a pipet and blowing it and a small quantity of air back into the suspension Incubation is carried out in a 37 C water bath and the mixing procedure is repeated after 2 hr After 4 hr a small droplet of the suspension is placed on a cover slip which is then inverted on a microscope slide that contains a droplet of half saturated crystal violet in 0 73 per cent NaCl solution An area in which the structure of the red cells is well preserved is selected for microscopic examination One hundred cells are examined and are classified as containing either five (or more) or four (or less) Heinz bodies With rare exceptions over 40 per cent of red cells from sensitive subjects contain five or more Heinz bodies Less than 30 per cent of red cells from nonanemic nonsensitive subjects contain this many Heinz bodies Before the blood of anemic patients is to be examined it is useful to centrifuge it slowly and to remove sufficient plasma to adjust the hematocrit to 40 per cent [*127*]

### GSH LEVELS

There is a highly significant difference between the average GSH level of sensitive and nonsensitive red cells [*41*] Some investigators initially attempted to use this measurement as a means for identifying sensitive subjects [*44 48 144*] Unfortunately there is too much overlap between the GSH levels of normal and sensitive red cells to make this a useful technique for the detection of drug sensitivity [*42*]

### GSH STABILITY TEST

The incubation of red cells with acetylphenylhydrazine and measurement of GSH stability is a useful method for the detection of the red cell defect of drug sensitivity [*42 130*] This test is somewhat more time consuming than the Heinz body method but can be performed relatively simply and rapidly It is considerably less sensitive to changes in oxygen tension and hematocrit than is the Heinz body procedure It gives clear separation of sensitive from nonsensitive subjects A recent report [*145*] suggests that this procedure is not reliable in detecting females who are sensitive to the in vivo hemolytic effects of primaquine but correlation between glucose 6-phosphate dehydrogenase assays and glutathione stability tests has been excellent in the hands of most investigators [*51, 54 55 141*] Only a few discrepancies between GSH stability tests and enzyme assays have been reported [*50 55 146*] The author regards this as the method of choice for survey and other routine purposes

Considerable confusion was caused originally by the observation that red cells obtained from infants up to 75 hr of age had unstable GSH [*54 63 146–148*] Zinkham [*146*] has clearly demonstrated that this in

stability is due to rapid depletion of already diminished quantities of blood gluco e Addition of glucose to the system rever es the apparent defect in all but a few infants who prove to have glucose 6 phosphate dehydrogenase deficiency and may therefore be regarded as truly sensitive [146 149]

**Procedure** [42] Venous blood is drawn into heparin or into ACD[1] solution The use of the latter anticoagulant a ures the presence of sufficient glucose

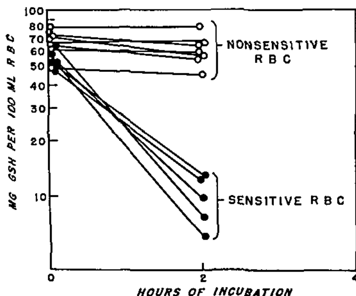



Fig 32 5 The results of the GSH stability test as carrie l out on the bloo l of seven n n nsitive an l five sen itive male subjects (F o 1 E B utle ( ) by permission of the Journal of Laboratory and Clinical Medicine )

as substrate for GSH reduction If ACD solution is used acceptable results may be expecte l even if sterile samples are stored for 4 we ks at 4 C [64 150] One milliliter of blood is added to a tube containing 5 mg acetyl phenylhydrazine These are mixed and shaken to ensure thorough oxygenation and incubated for 2 hr at 37 C At the end of the incubation period 2 ml distilled water is added Within a few minutes 5 ml of 3 per cent glacial metapho phoric acid (35 per cent $HPO_3$) is added with agitation followed by approximately 3 gm NaCl and the mixture is shaken Two milliliters of a filtrate is added to 6 ml of a saturated NaCl solution in a 25-mm cuvette with all solutions equilibrated at 20 to 22 C One milliliter of 2 per cent so lium nitropru side and 1 ml of a NaCN (0 07 M)–$Na_2CO_3$ (1 5 M) solution are added to develop color A pectrophotometer reading is made at 520 mμ within 1 min

After incubation with acetylphenylhydrazine the red cell GSH of ensitive ubjects is u ually less than 20 mg per 100 ml Non en itive red cells usually

[1] One l ter contains 16 0 gm sodium citrate 4 8 gm citric aci l 20 5 gm glucose One mill lit r of ACD is u ed per 5 ml whole blood

contain more than 35 mg GSH per 100 ml red cells (Fig 32 5) In most cases the difference in color is sufficient to permit correct interpretation with the naked eye [47] This test is unaffected by a wide variety of disease conditions [60 64]

## GLUCOSE 6-PHOSPHATE DEHYDROGENASE ASSAY

Demonstration of decreased activity of glucose 6-phosphate dehydrogenase is probably the most reliable and exact means for the detection of drug sensitive red cells It should be recalled that 6-phosphogluconate formed in the assays which have been used will be oxidized further if 6-phosphogluconic dehydrogenase is present This results in the reduction of additional TPN Unless a great excess of phosphogluconic dehydrogenase is present, and this does not appear to be true in red blood cells [150] the amount of TPN reduced will depend upon the level of both phosphogluconic dehydrogenase and glucose 6 phosphate dehydrogenase It must be remembered, therefore that abnormalities in the level of phosphogluconic dehydrogenase can also influence the results in GSSG linked TPN linked and dye-linked assays for glucose 6-phosphate dehydrogenase The latter enzyme is apparently quite stable when red cells are stored in ACD solution No decline in enzymatic activity has been noted in 4 weeks [151] The validity of results obtained on a single sample of red cells which showed a sudden drop in enzymatic activity on the seventh day of storage [140] is to be doubted

### *GSH linked Assay*

An assay for red cell glucose 6-phosphate dehydrogenase has been described in which the reduction of GSSG to GSH is measured by determining the amount of reduced glutathione in the mixture at various intervals after beginning incubation Glucose 6-phosphate dehydrogenase and glutathione reductase are supplied in the hemolyzate The former is the limiting factor in the reaction

**Procedure** [43] Red cells are washed in saline solution and hemolyzed in 5 vol distilled water for 1 hr at 4 C The hemolyzate is centrifuged at 1 400 g for 90 min (maximum temperature 37 C) The cleared hemolyzate is dialyzed for 12 hr at 4 C against 40 vol 0 067 *M* phosphate buffer pH 7 4 Incubation is carried out at 22 C in a 14-ml reaction mixture buffered to pH 7 4 by 0 036 *M* tris (hydroxymethyl)aminomethane) Oxidized glutathione is added to give a final concentration of $1 \times 10^{-}$ *M* TPN $1 \times 10^{-4}$ *M* glucose 6-phosphate $5 \times 10$ *M* and dialyzed hemolyzate 0 5 to 5 0 ml final volume to 14 ml with distilled water The reaction is stopped at the end of 15 min by the addition of metaphosphoric acid and reduced glutathione concentration is determined A modification [92] of this procedure has been described in which the hemolyzate is prepared in the cold The concentration of some of the reactants has been altered and the reaction is carried out at 37 C

*Spectrophotometric Measurement of the Reduction of TPN*

A more rapid method for the measurement of glucose 6 phosphate dehydrogenase activity in red cells is an adaptation of the procedure described earlier for the determination of this enzyme in other tissues [15?] It is based on the spectrophotometric measurement of the appearance of TPNH in a reaction mixture consisting of glucose 6-phosphate TPN buffer solution and hemolyzate (Fig 32 6)

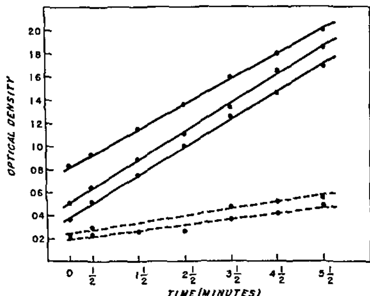



Fig 3 -6 Replicate direct spectrophotometric assays of glucose 6-phosphate dehydrogenase carried out on the red cells of one normal (——) and one sensitive (-- -) subject The assay method used was as described by Mark except that 0 5 ml 0 05 M glucose 6-phosphate was added as substrate instead of 0 5 M and that the total volume was increased to 3 ml Hemoglobin concentration in the two cuvettes was nearly the same It is apparent that TPN reduction is taking place at a more rapid rate in the preparation from erythrocytes of the normal subject than in the preparation from erythrocytes of the sensitive subject

**Procedure** [150] After removal of plasma and buffy coat red cells are washed twice in cold isotonic KCl at pH 7 4 and are lysed by freezing and thawing twice The assay system consists of 0 1 M $MgCl_2$ 0 5 ml 0 25 M glycylglycine buffer pH 7 6 0 5 ml ? 3 × 10⁻⁴ M TPN 0 1 ml 0 5 M* glucose 6-phosphate 0 5 ml hemolyzate water to 2 5 ml Activity is expressed as Δ O D (λ = 340 mμ) per min per gm Hb or per ml RBC or per 10 RBC

*The substitution of 0 05 M glucose 6-phosphate is satisfactory and much less expensive

*Dye linked Assay [125 153]*

A quick screening test has been described in which the decolorization (reduction) under oil of brilliant cresyl blue by a hemolyzate supplied with TPN and glucose 6-phosphate is observed with the naked eye Experience with this test is still limited

It appears certain that simpler screening methods for the detection of drug sensitive individuals will be developed and that more exact methods for quantitation of enzyme activity such as that of Glock and McLean [122] will be adapted to red cells for more precise studies

## GENETICS OF DRUG SENSITIVITY

The availability of in vitro methods for the detection of the red cell abnormality of drug sensitive subjects has made possible extensive studies of the genetics of this defect The mode of inheritance has been elucidated beautifully by Childs et al [126]

### DOMINANCE

The defect has been found in as many as three successive generations [74 126] (Fig 32 7 Family 12 LA) For this reason transmission as a recessive characteristic is extremely unlikely

### SEX DISTRIBUTION

Several curious features have come to light regarding the distribution of the erythrocyte defect between the sexes It was noted that severe GSH instability or severe glucose 6 phosphate dehydrogenase deficiency occurred most commonly among males [54 65 74 126] This appeared to support the clinical observation that severe hemolytic anemia on exposure to fava beans occurs more frequently among men than among women [47] It was also found that intermediate degrees of GSH instability occurred among *female relatives of sensitive subjects [54 74 126 154]* and that these females usually had intermediate degrees of enzyme deficiency [54, 66 74]

### GENETIC BASIS OF THE SEX DISTRIBUTION

It is apparent from these observations that the genetics of this defect *is not simple autosomal inheritance the defect* is either sex linked or sex limited The available pedigrees reveal that almost invariably it is the mother of affected males who carries the disorder if it is demonstrable in either parent [47 54 74 126] This finding makes it most probable that the defect is sex linked rather than sex limited [126] The latter possibility should not result in any alteration in the ratio of mothers and fathers of propositi affected

PENETRANCE

If the defect were transmitted as a sex linked *completely dominant* trait expression would be bimodal in distribution One would expect almost twice as many affected females as males and one parent of any affected individual would show evidence of the trait The e expectations are not borne out Intermediate degrees of GSH stability and enzyme

Fig 32 7 Some revealing pedigrees of families with primaquine sensitivity The K family only a part of which is presented and the C family were studied by Gross et al [ ] using an assay for glucose 6-phosphate dehydrogenase as the criterion for designation of subjects LA in Family 12 was studied by Childs et al [1 6] using the GSH stability test as a criterion of sensitivity Open symbols indicate subjects who had normal glucose 6-phosphate dehydrogenase activity or GSH stability tests Solid symbols indicate a well marked deficiency of glucose 6-phosphate dehydrogenase or marked GSH instability Half-shaded symbols indicate intermediate manifestations of the defect Symbols with a cross indicate that the subject was not examined

deficiency are evident among females [14 74 126 154] Furthermore the total of the intermediates and severely affected females is not twice that of males (Table 32 3) As indicated in Fig 32 7 affected subjects have been found in whom the defect could be demonstrated in neither parent when the criterion for classification was the GSH stability test (cf Family 12 LA III—7 8 produced IV—12)

It is thus apparent that the degree to which the presence of the gene expresses itself may differ considerably at least among females A simple explanation might be that a severe defect in a female indicates homozygosity while the intermediate defect indicates heterozygosity The

TABLE 32 3 INCIDENCE AND DISTRIBUTION OF DRUG SENSITIVITY

| Ra | S bg p | Typ f d t | N f s bj ts | I c d s f il ity p t ye | R fer |
|---|---|---|---|---|---|
| C i | B t h t ps | 8- mq | 1 398 | 0 1 | [18 156] |
| | S d m ns | 8 amq | 1 043 | 1 0 | [17] |
| | Punj b s | 8-amq | 00 | 13 5 | [27] |
| | Ind an tr ps | 8- mq | 1 915 | 0 3 | [16] |
| | Ashk s c J ws | | | | |
| | M l | GSH St | 203 | 0 | [74] |
| | F m l s | GSH St | 85 | 0 | [ 4] |
| | N n Ashk J w h l | GSH St | | | [74 149] |
| | Iraq | | 89 | 23 6 | |
| | Yem | | 6 | 8 1 | |
| | N th Afri a | | 43 | 2 3 | |
| | P si | | 9 | 0 | |
| | Egypt | | 19 | 0 | |
| | I d | | 1 | 0 | |
| | T k y | | 2 | 9 1 | |
| | B lg ri | | 9 | 0 | |
| | Oth d k wn | | 2 | 0 | |
| | N A hke J w l f l s | G H St | | | [74 149] |
| | I q | | 91 | 4 1 | |
| | Y m n | | 50 | 6 0 | |
| | N th Af | | 40 | | |
| | P a | | 9 | 44 4 | |
| | Egypt | | 19 | 0 | |
| | T k y | | 2 | 4 5 | |
| | B lg a | | 11 | 9 1 | |
| | Oth s a d kn wn | | 1 | 0 | |
| | Arabs | GSH St | 58 | 3 4 | [74 149] |
| | S d n ns | | | | |
| | M l s | GSH St | 61 | 13 1 | [156] |
| | F m l s | GSH St | 38 | 0 (10 5)† | [156] |
| | I d s | | | | |
| | M l s | Dye | 15 | 13 3 | [163] |
| | F m l s | Dy | 1 | 100 0 | [163] |
| | It l a (L g ri ) | GSH St | 100 | 0 | [156] |
| | N p fi d | | | | |
| | M l | GSH St | 30 | 0 | [42 117] |
| | F m l | GSH St | 71 | 0 | [42 117 122 ] |
| | N t p fied | G 6-pd | 153 | 1 3 | [157] |
| | N t p n d | 8 mq | 23 9 2 | 0 12 | [30 33 1 8 162] |
| Negro | S d ne ll s | 8- mq | 160 | 0 | [163] |
| | B t | GSH St | 100 | 2 0 | [164] |
| | Am N g oe | | | | |
| | Mal | 8 mq | 301 | 12 | [29 58 77 145 165] |
| | | GSH St | 308 | 11 | [49 11 122 14 ] |
| | F m l | 8 amq | 69 | 8 7 | [145] |
| | | G H St | 448 | 2 7 (7 6†) | [42 117 122 14 ] |
| | U pec fi d | G 6-pd | 152 | 7 | [157] |
| M g l | Ch | 8-amq | 38 | 5 3 | [11] |
| | M l | GSH St | 41 | 0 | [166] |
| | F m l | GSH St | 36 | 0 | [166] |
| | F m sa | 8 mq | 5 | 12 | [9] |
| | E t I d t | 8 amq | 9 | 11 | [11] |
| | Th —m l | GSH St | 9 | 11 | [166] |
| | J p se | | | | |
| | M le | GSH St | 6 | 0 | [166] |
| | Fem l | GSH St | 2 | 0 | [166] |
| I d n | N g n M k to | 8 mq | 200 | 0 | [167] |
| | P ns | | | | |
| | M l | Dy | 154 | 0 | [167 ] |
| | F m l | Dy | 74 | 0 | [167 ] |

Tl m thod by whi h n d f n t ty w d te m n d 8 mq = 8- m q l d g adm n t t w th h m ly d p t GSH St = gl t th tab l ty te t G 6 pd = ass y f red ll gl o 6-ph ph t d hyd g n Dy = dy l k d sc g t t

† I cl d g t m l at t

available pedigrees show clearly that this is not the case Thus some females with marked GSH instability and glucose 6 phosphate dehydrogenase deficiency must be heterozygous because they have borne boys who are not affected [cf Fig 32 7 Family K II—A K and E K have produced four negative sons (III—J K P K Go K and G K)] Further the fact that neither parent of some propositi has the defect demonstrable in vitro indicates that the gene can be carried without causing a detectable disorder of the red cell which is demonstrable in vitro Interestingly it has been found that the erythrocytes of at least some such individuals with a negative GSH stability test undergo hemolysis when primaquine is given [145] The glucose 6 phosphate dehydrogenase content of the red cells of such patients has not been reported

### GLUCOSE 6 PHOSPHATE DEHYDROGENASE ACTIVITY IN CELLS OTHER THAN ERYTHROCYTES

Because *glucose 6-phosphate dehydrogenase deficiency is genetically* determined it would appear reasonable that the synthesis of this enzyme might also be impaired in tissues other than the red blood cell Studies based on the intravenous administration of specifically labeled glucose show retarded appearance as $CO_2$ of the 1 carbon of glucose given to sensitive subjects and thus appear to support this supposition Only a very limited amount of direct data on enzyme activity in cells other than erythrocytes is available however Liver biopsies from two sensitive subjects showed less enzyme activity than those of four nonsensitive controls The difference was much smaller than that observed when red cell enzyme activities were compared The glucose 6-phosphate dehydrogenase activity of the leukocytes of 14 sensitive subjects did not differ appreciably from that of 31 controls [*126c*] Blood platelets from sensitive subjects in contrast have a marked reduction in glucose 6 phosphate dehydrogenase activity [*126b*] These findings are compatible with the concept that the enzyme produced by sensitive subjects is abnormal and is more susceptible to damage than normal enzyme [*125*] Nucleated cells such as leukocytes and liver cells could maintain enzyme activity at normal or near normal levels by continued resynthesis but non nucleated cells such as platelets and red cells unable to synthesize more enzyme as they aged would become progressively enzyme-depleted

## INCIDENCE AND DISTRIBUTION OF DRUG SENSITIVITY

Two types of information are available on which to base estimates of the incidence and distribution of glucose 6-phosphate dehydrogenase deficiency The first is the evaluation of reports in the literature of acute hemolytic anemia following the ingestion of drugs The second consists of

population surveys using the methods developed for in vitro detection of the red cell abnormality of sensitive individuals

Some of the more significant information is summarized in Table 32 3 The highest incidence in a single group is in a relatively small series of Iraqi Jews Twenty per cent of this population has the defect Other groups of Sephardic Jews have a lower but still quite appreciable incidence High incidence has been consistently reported in American Negroes among whom the gene frequency seems to be approximately 10 per cent judging from incidence of positive in vitro tests and incidence of hemolysis in males Presumably the incidence would be similarly high in West Africa A positive GSH stability test result has been demonstrated in a native Nigerian woman presently a student in the United States [117] The failure to observe hemolysis among Sudanese villagers given pamaquine and the 2 per cent incidence of positive GSH stability test results among Bantu natives lead one to conclude that the incidence of the defect is lower in certain parts of Africa The incidence of the defect is appreciable in India Formosa and perhaps Thailand, but lower among the Chinese Information about the distribution of the defect but not its incidence may be obtained from a recent exhaustive review of the distribution of favism by Sansone et al [47] In addition to numerous reports from Sardinia cases have been reported from almost every region in Italy, from Greece Israel, Lebanon, Turkey Spain Bulgaria and even from West China

Because the distribution of the defect parallels roughly the distribution of malaria, it has been suggested that glucose 6 phosphate dehydrogenase deficiency like sickle cell hemoglobin may confer resistance to malaria [153] Preliminary studies appear to support this point of view [167b]

## A CLOSELY RELATED DEFECT

When drug sensitive erythrocytes are not challenged with drug their survival is normal Observations by Newton et al [168 169] confirmed by others [170 171] suggest that certain subjects who clinically are suffering from nonspherocytic hemolytic anemia have the same enzymatic abnormality as drug sensitive subjects In common with drug sensitive subjects their red cells have unstable GSH and have markedly diminished glucose 6-phosphate dehydrogenase activity Heinz body formation in their red cells after incubation with acetylphenylhydrazine is also identical with that seen in drug sensitive subjects They differ from drug sensitive subjects in that shortening of red cell survival as measured by the $Cr^{51}$ technique occurs in these subjects and in their mothers [169 171] Furthermore this disorder differs from drug sensitivity in that all the individuals affected have been Caucasian (in one case of Irish stock)

Other cases of hemolytic anemia reported recently [133] may fit into

this category It seems most likely that this form of nonspherocytic hemolytic anemia represents the effect of another much rarer genetic mutation which also affects glucose 6 phosphate dehydrogenase activity, but perhaps through another mechanism and perhaps to a much greater degree

## SUMMARY

1 When certain ordinarily harmless drugs are administered to susceptible persons an acute hemolytic reaction results Among these are 8 aminoquinoline antimalarials such as primaquine certain sulfonamides acetanilid phenacetin Furadantin and many others Persons with a history of favism have the same red cell abnormality

2 When sensitive persons receive the drug the older members of the red cell population are rapidly destroyed The hemolytic anemia then ceases even while drug administration is continued because young erythrocytes are resistant to destruction

3 Susceptibility to drug induced hemolysis is due to an intrinsic abnormality of the erythrocyte

4 Drug sensitive red cells have reduced glucose 6-phosphate dehydrogenase activity This enzyme normally catalyzes the oxidation of glucose 6-phosphate which is coupled to reduction of TPN TPNH in turn maintains glutathione in its reduced form (GSH) Drug sensitive red cells are unable to reduce TPN at a normal rate Probably as a result of this enzymatic defect the GSH content of sensitive cells is decreased and when sensitive cells are challenged with drug in vivo or in vitro a further decrease in GSH concentration takes place GSH may be an important agent in maintaining structural integrity of the red cell as may be TPNH but the ultimate way in which glucose 6-phosphate dehydrogenase deficiency is linked to red cell drug susceptibility is unknown

5 Drug sensitive red cells can be detected in vitro by assaying them for glucose 6-phosphate dehydrogenase activity They can also be detected by incubating red cells under standardized conditions with acetylphenylhydrazine and by measuring the reduced glutathione content or by observing the pattern of Heinz body formation

6 The red cell abnormality of drug sensitivity is an inherited sex linked dominant trait Marked variability in the degree of penetrance is observed among affected females who are heterozygotes The defect is widely distributed it is common among American Negroes Sephardic Jews and Sardinians

7 While red cells from patients with the defect have normal life spans unless challenged by drug a similar biochemical abnormality has also been described in a few patients with nonspherocytic hemolytic anemia

# BIBLIOGRAPHY

1 Muhlens P Die Behandlung der natürlichen menschlichen Malaria-infektion mit Plasmochin Naturwissenschaften **14** 1162 1926
2 Cordes W Experiences with plasmochin in malaria (preliminary reports) in *15th Annual Report* pp 66–71 United Fruit Co (Medical Department) 1926
3 Cordes W Observations on the toxic effect of plasmochin in *16th Annual Report* pp 62–67 United Fruit Co (Medical Department) 1927
4 Liselberg K P Plasmochinvergiftung Wien klin Wchnschr **40** 595 1927
5 Brosius O T Plasmochin in malaria in *16th Annual Report* pp 26–53 United Fruit Co (Medical Department) 1927
6 Brosius O T Plasmochin in malaria in *17th Annual Report* pp 51–70 United Fruit Co (Medical Department) 1928
7 Cordes W Zwischenfalle bei der Plasmochinbehandlung Arch Schiffs u Tropen Hyg **32** 143 1928
8 Palma M D Considerzioni sulla plasmochinoterapia della malaria Riforma med **44** 753 1928
9 Namikawa H Symptoms of poisoning in the treatment of malaria Taiwan Igakkai Zasshi **284** 75 1928
10 Menk W Combined quinine and plasmochin treatment for malaria in Haitian Negroes in *16th Annual Report* pp 78–81 United Fruit Co (Medical Department) 1927
11 Roskott E R A L and Seno R Ervaringen met plasmochin Geneesk Tijdschr Ned Indie **68** 80 1928
12 Kligler I J and Reitler R Studies in malaria IV Prophylactic use of plasmochin in a Bedouin population Riv malariol **8** 28 1929
13 Manai A Ittero da plasmochina Policlinico (sez prat) **36** 1215 1929
14 Baermann G and Smits E Über Plasmochin II Mitteilung Arch Schiffs u Tropen Hyg **33** 24 1929
15 Freiman M Plasmoquine and plasmoquine compound in the treatment of malaria J Trop Med Hyg **32** 165 1929
16 Manifold J A Report on a trial of plasmoquine and quinine in the treatment of benign tertian malaria J Roy Army M Corps **56** 321 1931
17 Missiroli A and Marino P Anwendung des Chinoplasmin zur Malariasanierung Arch Schiffs u Tropen Hyg **38** 1 1934
18 Amy A C Hemoglobinuria a new problem on the Indian frontier J Roy Army M Corps **62** 178 269 318 1934
19 Ficacci L Emoglobinuria da plasmochina Policlinico (sez prat) **42** 136 1935
20 Sein M Case of hemoglobinuria caused by plasmochin taken as prophylactic against malaria Indian M Gaz **72** 86 1937
21 Mann W N Haemoglobinuria following the administration of plasmoquine Tr Roy Soc Trop Med Hyg **37** 151 1943
22 Smith S Note regarding hemoglobinuria following the administration of plasmochin Tr Roy Soc Trop Med Hyg **37** 155 1943
23 Braun K and de Vries A Plasmochin and quinine as the cause of acute haemolytic anemia Harefuah **27** 219 1944
24 West J B and Henderson A B Plasmochin intoxication Bull U S Army M Dept **82** 87 1944
25 Swantz H E and Bayliss M Hemoglobinuria Report of ten cases of its occurrence in Negroes during convalescen e from malaria War Med **7** 104 1945
26 Hardgrove M and Appelbaum I L Plasmochin toxicity Analysis of 258 cases Ann Int Med **25** 103 1946
27 Dimson S B and McMartin R B Pamaquine haemoglobinuria Quart J Med **15** 25 1946
28 Loeb R F Activity of a new antimalarial agent pentaquine (SN 13 276) Statement approved by the Board for Coordination of Malarial Studies J A M A **132** 321 1946

29 Feldman H A Lacker H Murphy F D and Watson R B Pamaquine naphthoate as a prophylactic for malarial infections J Clin Invest 26 77 1947
30 Atchley J A Yount E H Husted J R Pullman T N Alving A S and Eichelberger L Reactions observed during treatment with pentaquine administered with quinacrine (Atabrine) metachloridine (SN 11437) and with sulfadiazine J Nat Malaria Soc 7 118 1948
31 Turchetti A Forme poco frequenti di emoglobinuria da farmaci in corso di infezione malarica Riforma med 62 3 5 1948
32 Keng K L Een geval van zwartwaterkoorts met cyanose (methaemoglobinaemia) waarschijnlijk door plasmochine Med Maandbl 1 342 1948
33 Earle D P Jr Bigelow F S Zubrod C G and Kan C A Studies on the chemotherapy of the human malarias IX Effect of pamaquine on the blood cells of man J Clin Invest 27 121 Suppl 1948
34 Mer G Birnbaum D and Kligler I J Lysis of blood of malaria patients by bile or bile salts Tr Roy Soc Trop Med Hyg 34 373 1940–1941
35 Coatney G R Cooper W G Eyles D E Culwell W B White W C and Lints H A Studies in human malaria XXVII Observations on the use of pentaquine in the prevention and treatment of Chesson strain vivax malaria J Nat Malaria Soc 9 2?? 1950
36 Zylmann G *In vitro* and *in vivo* Versuche uber die haemolytischen Eigenschaften der synthetischen Malariamittel D utsch Tropenmed Ztschr 48 7 1944
37 Edgcomb J H Arnold J Yount E H Alving A S and Eichelberger L Primaquine (SN 13272) a new curative agent in vivax malaria a preliminary report J Nat Malaria Soc 9 284 1950
38 Dern R J Weinstein I M LeRoy G V Talmage D W and Alving A S The hemolytic effect of primaquine I The localization of the drug induced hemolytic defect in primaquine-sensitive individuals J Lab Clin Med 43 303 1954
39 Beutler E Dern R J and Alving A S The hemolytic effect of primaquine IV The relationship of cell age to hemolysis J Lab Clin Med 44 439 1954
40 Dern R J Beutler E and Alving A S The hemolytic effect of primaquine V Primaquine sensitivity as a manifestation of a multiple drug sensitivity J Lab Clin Med 46 30 1955
41 Beutler E Dern R J Flanagan C L and Alving A S The hemolytic effect of primaquine VII Biochemical studies of drug-sensitive erythrocytes J Lab Clin Med 45 286 1955
42 Beutler E The glutathione instability of drug-sensitive red cells A new method for the *in vitro* detection of drug sensitivity J Lab Clin Med 49 84 1957
43 Carson P E Flanagan C L Ickes C E and Alving A S Enzymatic deficiency in primaquine-sensitive erythrocytes Science 124 484 1956
44 Sansone G and Segni G Prime determinazione del glutatione (GSH) ematico nel favismo Boll soc ital biol sper 32 456 1956
45 Sansone G and Segni G L Instabilita del glutathione ematico (GSH) nel favismo utilizzazione di un test sel ttivo Introduzione al problema genetico Boll soc ital biol sper 33 1057 1957
46 Sansone G and Segni G Nuovi aspetti dell alterato bio-chimismo degli eritrociti di favici assenza pressoche completa della glucoso-6-p deidrogenase Boll soc ital biol sper 35 3 7 1958
47 Sansone G Piga A M and Segni G *Il Favismo* Edizioni Minerva med ca Torino 1958
48 Szeinberg A Sheba C Hirshorn N and Bodonyi E Studies on erythrocytes in cases with past history of favism and drug induced acute hemolytic anemia *Blood* 12 603 1957
49 Szeinberg A Asher Y and Sheba C Studies on glutathione stability in erythrocytes of cases with past history of favism or sulfa-drug induced hemolysis Blood 13 348, 1958
50 Szeinberg A Sheba C and Adam A Enzymatic abnormality in erythrocytes of a population sensitive to *Vicia faba* or drug induced hemolytic anemia, Nature London 181 1256 1958
51 Zinkham W H Lenhard R. L. Jr and Childs B A deficiency of glucose-6-

phosphate dehydrogenase activity in erythrocytes from patients with favism Bull Johns Hopkins Hosp 102 161 1958
52 Panizon F Sul comportamento delle emazie dei fabici di fronte all idrossilamina *in vitro* Studi sassaresi 36 164 195
53 Panizon F and Lujatti G Studio sul fluctuatione eritrocitario nel favismo Acta paediat latina (Parma) 11 71 1958
54 Gross R T Hurwitz R E and Marks P A An hereditary enzymatic defect in erythrocyte metabolism glucose-6-phosphate dehydrogenase deficiency J Clin Invest 37 1176 1958
55 Larizza P Brunetti P Grignani F and Ventura S I Individualita bioenzimatica dell eritrocite fabico sopra alcune anomalie biochemiche ed enzimatiche delle emazie nei pazienti affetti da favismo e nei loro familiari Haematol 43 205 1958
56 Sartori E and Panizon L Nuove prospettive nello studio del favismo Studi sassaresi 35 363 1957
57 Larizza P Brunetti P Grignani F and Ventura S I fabici sono sensibili alla primachina Minerva med 49 3769 1958
58 Hockwald R S Arnold J Clayman C B and Alving A S Status of primaquine IV Toxicity of primaquine in Negroes J A M A 149 1568 1952
59 Beutler E Dern R J and Alving A S Studies of the hemolytic anemia induced by primaquine and related compounds Abstracts of Papers Presented at the American Chemical Society Division of Medicinal Chemistry in New York N Y Sept 12 to 17 1954
60 Beutler E The hemolytic effect of primaquine and related compounds a review Blood 14 103 1959
61 Kimbro E L Jr Sachs M V and Torbet J V Mechanism of the hemolytic anemia induced by nitrofurantoin (furadantin) Bull Johns Hopkins Hosp 101 245 1957
62 Kellermeyer R W Tarlov A R Schrier S L and Alving A S Hemolytic effect of commonly used drugs on erythrocytes deficient in glucose-6-phosphate dehydrogenase J Lab Clin Med 52 827 1958 (abstract)
63 Zinkham W H and Childs B A defect in GSH metabolism in erythrocytes from patients with a naphthalene induced hemolytic anemia Pediatrics 22 461 1958
64 McGovern J J Isselbacher K Rose P J and Grossman M S Observations on the glutathione (GSH) stability of red blood cells A M A J Dis Child 96 502 1958
65 Dawson J P Thayer W and Desforges J F Acute hemolytic anemia in the newborn infant due to naphthalene poisoning report of two cases with investigations into the mechanism of the disease Blood 13 1113 1958
66 Sansone G and Segni G Difetto biochimico eritrocitario a carattere genetico in un bambino con anemia emolitica acuta da naftalina Boll soc ital biol sper 34 516 1958
67 Sansone G Segni G Difetto biochimico eritrocitario a carattere genetico in un bambino con anemia emolitica acuta da naftalina Boll Soc ital biol sper 34 615 1958
68 Szeinberg A Sheba C Hirschorn N and Bodonyi E Chemical studies on erythrocytes in cases of haemolytic anemia due to *Vicia faba* sulphonamides and p-aminosalicylic acid Bull Res Council Israel 6E 115 1956
69 Dern R J Beutler E and Alving A S The hemolytic effect of primaquine II The natural course of the hemolytic anemia and the mechanism of its self limited character J Lab Clin Med 44 171 1954
70 Beutler E Dern R J and Alving A S The hemolytic effect of primaquine III A study of primaquine-sensitive erythrocytes J Lab Clin Med 44 177 1954
71 Zuelzer W W and Apt L Acute hemolytic anemia due to naphthalene poisoning J A M A 141 185 1949
72 West M and Zimmerman H J Hemolytic anemia in patient receiving nitrofurantoin (furadantin) J A M A 162 637 1956

73 Luisada L Favism singular disease affecting chiefly red blood cells Medicine 20 229 1941

74 Szeinberg A Sheba C and Adam A Selective occurrence of glutathione instability in red blood corpuscles of the various Jewish tribes Blood 13 1043 1958

74a Greenberg M S and Wong H The influence of fava beans and primaquine on glutathione unstable red blood cells in vitro Clin Res 7 205 1959

75 Sansone G and Segni G L Anemia emolitica acuta da sulfamide e quella da PAS differenze patogenetiche Boll soc ital biol sper 34 1536 1958

76 Harris J W Studies on the mechanism of a drug induced hemolytic anemia J Lab Clin Med 47 760 1956

77 Jones R Jr Jackson L S DiLorenzo A Marx R L Levy B L Kenny E C Gilbert M Johnston M N and Alving A S Korean vivax malaria III Curative effect and toxicity of primaquine in doses from 10 to 30 mg daily Am J Trop Med Hyg 2 977 1953

78 Granick S The chemistry and functioning of the mammalian erythrocyte Blood 4 404 1949

79 Bartlett G R and Marlow A A Enzyme systems in the red blood cell Bull Scripps Metabol Clin 2 1 1951

80 Dimant E Landsberg E and London I M The metabolic behavior of reduced glutathione in human and avian erythrocytes J Biol Chem 213 769 1955

81 Elder H A and Mortensen R A The incorporation of labeled glycine into erythrocyte glutathione J Biol Chem 218 261 1956

82 Mortensen R A Haley M I and Elder H A The turnover of erythrocyte glutathione in the rat J Biol Chem 218 269 1956

82a Leder I S and Handler P Synthesis of nicotinamide mononucleotide by human erythrocytes in vitro J Biol Chem 189 889 1951

82b Tulpule P G Species difference in pyridine nucleotide synthesis by erythrocytes Nature London 181 1801 1958

83 Altman K I The in vitro incorporation of a C-14-acetate into the stroma of erythrocytes Arch Biochem 42 478 1953

84 James A T Lovelock J E and Webb J The biosynthesis of fatty acids by human red blood cells Biochem J 66 60 1957

84a O Donnell V J Ottolenghi P Malkin A Denstedt O F and Heard R D H The biosynthesis from acetate 1 C of fatty acids and cholesterol in formed blood elements Canad J Biochem Physiol 36 1125 1958

84b Marks P A and Gellhorn A Lipid synthesis in human leucocytes and erythrocytes in vitro Fed Proc 18 281 1959

85 Harrop G and Barron E Effect of methylene blue and other dyes on the oxygen consumption of mammalian and avian erythrocyte J Exper Med 48 207 1928

86 Brin M and Yonemoto R H Stimulation of the glucose oxidative pathway in human erythrocytes by methylene blue J Biol Chem 230 307 1958

87 Gibson Q H The reduction of methaemoglobin in red blood cells and studies on the cause of idiopathic methaemoglobinaemia Biochem J 42 13 1948

88 Dickens F Recent advances in knowledge of the hexose monophosphate shunt Ann New York Acad Sc 75 71 1958

89 Dische Z Some regulatory factors in the interconversion of glucose-6-phosphate and ribose-5-phosphate in human erythrocytes Ann New York Acad Sc 75 129 1958

90 Rall T W and Lehninger A L Glutathione reductase of animal tissues J Biol Chem 194 119 1952

91 Francoeur M and Denstedt O F Metabolism of mammalian erythrocytes VII The glutathione reductase of the mammalian erythrocyte Canad J Biochem Physiol 32 663 1954

92 Schrier S L Kellermeyer R W Carson P E Ickes C E and Alving A S The hemolytic effect of primaquine IX Enzymatic abnormalities in primaquine-sensitive erythrocytes J Lab Clin Med 52 109 1958

93 Mills G C Hemoglobin catabolism I Glutathione peroxidase an erythrocyte

enzyme which protects hemoglobin from oxidative breakdown J Biol Chem 229 189 1957
94 Mills G C and Randall H P Hemoglobin catabolism II The protection of hemoglobin from oxidative breakdown in the intact erythrocyte J Biol Chem 232 589 1958
95 Fegler G Relationship between reduced glutathione content and spontaneous haemolysis in shed blood Nature London 170 624 1952
96 Klebanoff S J Glutathione metabolism 2 The oxidation and reduction of glutathione in intact erythrocytes Biochem J 65 423 1957
97 Huennekens F M Caffrey R W Basford R E and Gabrio B W Erythrocyte metabolism IV Isolation and properties of methemoglobin reductase J Biol Chem 227 261 1957
98 Talalay P and Williams Ashman H G Activation of hydrogen transfer between pyridine nucleotides by steroid hormones Proc Nat Acad Sc 44 15 1958
99 Horecker B L and Hiatt H H Pathways of carbohydrate metabolism in normal and neoplastic cells New England J Med 258 177 1958
100 Wieland T Chemistry and properties of glutathione *Glutathione* p 45 Proceedings of the Symposium held at Ridgefield Conn November 1953 Academic Press Inc New York 1954
101 Calvin M Mercaptans and disulfides some physics chemistry and speculation in *Glutathione* p 3 Proceedings of the Symposium held at Ridgefield Conn November 1953 Academic Press Inc New York 1954
102 Lyman C M and Barron E S G Studies on biological oxidations VIII The oxidation of glutathione with copper and hemochromogens as catalysts J Biol Chem 121, 275 1937
103 Tsen C C and Tappel A L Catalytic oxidation of GSH and other sulfhydryl compounds by selenite J Biol Chem 233 1230 1958
104 Eggleton P and Fegler G Reduced glutathione and spontaneous methaemoglobin formation in haemolysates Quart J Exper Physiol 37 163 1952
105 Borsook H Davenport H W Jeffreys C E P and Warner R C The oxidation of ascorbic acid and its reduction *in vitro* and *in vivo* J Biol Chem 117 237 1937
106 Woodward G E and Fry E G The determination of blood glutathione J Biol Chem 97 465 1932
107 Mason H L Research Club of the Mayo Foundation abstracts of papers presented at the meeting on Mar 13 1931 Proc Staff Meet Mayo Clin 6 168 1931
108 Racker E The mechanism of action of glyoxalase J Biol Chem 190 685 1951
109 Fujita A and Numata I Über die jodometrische Bestimmung des Glutathions in Geweben II Mitteilung Bestimmung des Gesamtglutathions Biochem Ztschr 299 262 1939
110 Dohan J S and Woodward W E Electrolytic reduction and determination of oxidized glutathione J Biol Chem 129 393 1939
111 Meldrum N U and Tarr H L A XV The reduction of glutathione by the Warburg Christian system Biochem J 29 108 1934
112 Anderson D G Stafford H A Conn E E and Vennesland B The distribution in higher plants of triphosphopyridine nucleotide linked enzyme systems capable of reducing glutathione Plant Physiol 27 675 1952
113 Langdon R G Properties and mechanism of action of purified glutathione reductase Biochim et Biophys acta 30 432 1958
114 Carson P Schrier S and Flanagan C L Use of DPNH as coenzyme for glutathione reductase of hemolyzates Fed Proc 16 19 1957 (abstract)
115 Nechcles T and Beutler E Unpublished observations
116 Beutler E Robson M and Buttenwieser E The mechanism of glutathione destruction and protection in drug sensitive and non sensitive erythrocytes *In vitro* studies J Clin Invest 36 617 1957
117 Beutler E Unpublished observations

118 Benesch R F and Benesch R Relation between erythrocyte integrity and sulfhydryl groups Arch Biochem 48 38 1954

119 Sheets R F Hamilton H E and DeGowin L I Hemolysis of human erythrocytes by a sulfhydryl inhibitor *p* chloromercuribenzoic acid Proc Soc Exper Biol & Med 91 4 3 1956

120 Jowett M and Quastel J H LXX The glyoxalase activity of the red blood cell The function of glutathione Biochem J 27 486 1933

121 Barron E S G and Singer T P Enzyme systems containing active sulfhydryl groups The role of glutathione Science 97 356 1943

122 Glock G L and McLean P Further studies on the properties and assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver Biochem J 55 400 1953

122a Motulsky A Kraut J M Thiems W T and Musto D F Biochemical genetics of glucose-6-phosphate dehydrogenase deficiency Clin Res 7 89 1959 (abstract)

123 Johnson A B and Marks P A Glucose metabolism and oxygen consumption in normal and glucose 6-phosphate dehydrogenase deficient human erythrocytes Clin Res 6 187 1958 (abstract)

123a Marks P A and Gross R T Drug induced hemolytic anemias and congenital galactosemia Bull New York Acad Med 35 433 1959

124 Carson P E Schrier S L and Alving A S Inactivation of glucose-6-phosphate dehydrogenase in human erythrocytes J Lab Clin Med 48 794 1956 (abstract)

125 Kirkman H N Characterization of partially purified glucose-6-phosphate dehydrogenase from normal and primaquine-sensitive erythrocytes Fed Proc 18 261 1959

126 Childs B Zinkham W Browne E A Kimbro E L and Torbert J V A genetic study of a defect in glutathione metabolism of the erythrocyte Bull Johns Hopkins Hosp 102 21 1958

126a Marks P A and Gross R T Erythrocyte glucose-6-phosphate dehydrogenase deficiency evidence of differences between Negroes and Caucasians with respect to this genetically-determined trait J Clin Invest 38 2253 1959

126b Ramot B Szeinberg A Adam A Sheba C and Gafni D A study of subjects with erythrocyte glucose 6-phosphate dehydrogenase deficiency investigation of platelet enzymes J Clin Invest 38 1659 1959

126c Marks P A Gross R T and Hurwitz R E Gene action in erythrocyte deficiency of glucose-6-phosphate dehydrogenase tissue enzyme-levels Nature London 183 1266 1959

127 Flanagan C L Schrier S L Carson P E and Alving A S The hemolytic effect of primaquine VIII The effect of drug administration on parameters of primaquine sensitivity J Lab Clin Med 51 600 1958

128 Szeinberg A and Chari Bitron A Blood GSH concentration after hemolytic anemia due to *Vicia faba* or sulphonamides Acta haematol 18 229 1957

129 Woodward G E Glyoxalase III Glyoxalase as a reagent for the quantitative micro-estimation of glutathione J Biol Chem 109 1 1935

130 Beutler E *In vitro* studies of the stability of red cell glutathione a new test for drug sensitivity J Clin Invest 35 690 1956 (abstract)

131 Kalaw E and Desforges J F The mechanism of action of hemolysis-inducing drugs on glucose-6-phosphate dehydrogenase Clin Res 7 11 1959 (abstract)

132 LeRoy G V Okita G and Tocus E C Personal communication

133 Waller H D Löhr G W and Tabatabai M Hämolyse und Fehlen von Glucose-6-phosphatdehydrogenase in roten Blutzellen (eine Fermentanomalie der Erythrocyten) Klin Wchnschr 35 10 1957

134 Löhr G W and Waller H D Hämolytische Erythrocytopathie durch Fehlen von Glukose-6-phosphatdehydrogenase in roten Blutzellen, eine dominant vererbliche Krankheit Klin Wchnschr 36 865 1958

135 Schrier S L Kellermeyer R W and Alving A S Coenzyme studies in primaquine-sensitive erythrocytes Proc Soc Exper Biol & Med 99 354 1958

136 Schrier, S L Kellermeyer R W Carson P E Ickes C E and Alving A S The hemolytic effect of primaquine X Aldolase and glyceraldehyde-3-phosphate dehydrogenase activity in primaquine-sensitive erythrocytes J Lab Clin Med 54 232 1959
136a Heller I and Weinstein H G Aldolase isocitric dehydrogenase and malic dehydrogenase in glucose-6-phosphate dehydrogenase deficient erythrocytes J Lab Clin Med 54 824 1959
137 Bartlett G R Organization of red cell glycolytic enzymes cell coat phosphorus transfer Ann New York Acad Sc 75 110 1958
138 Rubinstein, D Ottolenghi P and Denstedt O F The metabolism of the erythrocyte XIII Enzyme activity in the reticulocyte Canad J Biochem Physiol 34 222 1956
139 Marks P A Johnson A B and Hirschberg E Effect of age on the enzyme activity in erythrocytes Proc Nat Acad Sc 44 529 1958
140 Löhr G W Waller H D Karges O Schlegel B and Müller A A Zur Biochemie der alterung Menschlicher Erythrocyten Klin Wehnschr 36 1008 1958
141 Sansone G Borrone C and Rovei S Suscettiblità degli eritrociti a formare corpi di Heinz *in vitro* in condizioni normali (neonati e lattanti) e patologiche (favismo e talassemia) Boll soc ital biol sper 34 1561 1958
141a Grignani F and Brunetti P Carenza di glucosio 6-fosfato deidrogenasi nelle emazie dei fabici nel corso della crisi emolitica ed a distanza dalla stessa Rass med Sarda 60 399 1958
142 Beutler E Dern R J and Alving A S The hemolytic effect of primaquine VI An *in vitro* test for sensitivity of erythrocytes to primaquine J Lab Clin Med 45 40 1955
143 Webster S H Heinz body phenomenon in erythrocytes A review Blood 4 479 1949
144 Carcassi U Lenzerini L and Contu L Il glutatione eritrocitario nel favismo ittero-emoglobinurico Arch ital sci med trop e parassitol 38 335 1957
145 Alving A S Kellermeyer R W Tarlov A Schrier S and Carson P E Biochemical and genetic aspects of primaquine-sensitive hemolytic anemia Ann Int Med 49 240 1958
146 Zinkham W H An *in vitro* abnormality of glutathione metabolism in erythrocytes from normal newborn mechanism and clinical significance Pediatrics 23 18 1959
147 Szeinberg A Ramot B Adam A and Sheba C Glutathione metabolism in cord blood and in the newborn infant A M A J Dis Child 96 ... 1958
148 Gross R D and Hurwitz R L The pentose phosphate pathway in human erythrocytes Relationship between age of the subject and enzyme activity Pediatrics 22 453 1958
149 Szeinberg A Ramot B Sheba C Adam A Halbrecht I Rikover M Vishniewsky S and Rabau E Glutathione metabolism in cord and newborn infant blood (to be published)
150 Szeinberg A Sheba C and Adam A Storage stability of blood with instable glutathione Israel Med J 17 265 1958
151 Kornberg A and Horecker B L Glucose 6-phosphate dehydrogenase in *Methods in Enzymology* edited by S P Colowick and N O Kaplan vol 1 p 323 Academic Press Inc New York 1955
152 Marks P A Red cell glucose-6-phosphate and 6-phosphogluconic dehydrogenases and nucleoside phosphorylase Science 127 1338 1958
153 Motulsky A G and Campbell J M A screening test for glucose-6-phosphate dehydrogenase deficiency of the red cell suitable for genetic surveys Possible relationship of the enzyme defect of malaria Blood (in press)
154 Sansone G and Segni G Sensitivity to broad beans Lancet 2 295 1957
155 Dixon H B F A report on six hundred cases of malaria treated with plasmoquine and quinine J Roy Army M Corps 60 431 1933

156 Sansone G Segni G and DeCecco C Il difetto biochimico eritrocitario predisponente all emolisi favica Prime ricerche sulla popolazione ligure e su quella sarda Boll soc ital biol sper 34 1558 1958
157 Gross R T Marks P A Hurwitz R E Beasley J and Sui M Studies on the hereditary enzymatic defect in certain drug induced hemolytic anemias A M A J Dis Child 96 456 1958
158 Most H Kane C A Lavietes I H London I M Schroeder E F and Hayman J M Jr Combined quinine plasmochin treatment of vivax malaria effect on relapse rate Am J M Sc 212 550 1946
159 Warthin T A Levine S A and Evans R R Malaria Observations on treatment with chloroquine (SN 7618) and combined quinine and plasmochin New England J Med 238 467 1948
160 Alving A S Pentaquine (SN 13 276) and Isopentaquine (SN 13 274) therapeutic agents effective in reducing relapse rate in vivax malaria *Proceedings of the Fourth International Congress on Tropical Medicine and Malaria* Washington D C May 10–18 1948
161 Clayman C B Arnold J Hockwald R S Young E H and Edgcomb J H Status of primaquine III Toxicity of primaquine in Caucasians J A M A 149 1563 1952
162 Gennis J Straus B Kenney M and Klein B The use of primaquine for the treatment of malaria in Korean veterans Am J Med 17 ? 3 1951
163 Henderson L H Prophylaxis of malaria in the Sudan with special reference to the use of plasmoquine Tr Roy Soc Trop Med & Hyg 28 157 1934
164 Bothwell T H and Charlton R J Personal communication
165 Dern R J Beutler E and Alving A S Unpublished observations (cited in [60])
166 Beutler E Yeh M and Necheles T Incidence of the erythrocyte defect associated with drug-sensitivity among Oriental subjects Nature 183 684 1959
167 Thaeler A D Jr Arnold J and Alving A S A clinical study of primaquine (SN 13 272) in the treatment of malaria among the Miskito Indians of Nicaragua Am J Trop Med Hyg 2 989 1953
167a Best W R Absence of erythrocyte glucose 6-phosphate dehydrogenase deficiency in certain Peruvian Indians J Lab Clin Med 54 791 1959
168 Motulsky A Personal communication
168 Newton W A Jr and Bass J C Glutathione sensitive chronic non-spherocytic hemolytic anemia A M A J Dis Child 96 501 1958
169 Newton W A Jr and Frajala W J Drug sensitive chronic hemolytic anemia Family studies Clin Res 6 392 1958 (abstract)
170 Zinkham W H and Lenhard R E Metabolic abnormalities of erythrocytes from patients with congenital non-spherocytic hemolytic anemia J Pediat 55 319 1959
171 Shahidi N T and Diamond L K Enzyme deficiency in erythrocytes in congenital non spherocytic hemolytic anemia Pediatrics 24 245 1959

# Chapter 33

# The Hereditary Methemoglobinemias*

*Park S Gerald*

## DEFINITION

Hereditary methemoglobinemia or hereditary methemoglobinemic cyanosis is a rare condition More than one hundred cases have been reported in the literature The disease has been described in Caucasians Eskimos and Chinese Many of the patients have been of Greek stock

The major feature of the disorder is a diffuse persistent slate gray cyanosis which is present from early infancy and unassociated with clubbing of the fingers or significant cardiac murmurs Methemoglobin comprises 20 to 50 per cent of the total hemoglobin Compensatory polycythemia and associated reticulocytosis are occasionally observed The disease is frequently well tolerated and patients may reach an advanced age Cardiac and respiratory distress are conspicuously absent although exertion may cause dyspnea palpitation easy fatigability, and severe headache The conjunctival and retinal veins may be congested

The blood has a chocolate hue and fails to turn a normal red color upon oxygenation This is attributable to the presence in the erythrocytes of excessive amounts of methemoglobin (ferrihemoglobin) an oxidation product of hemoglobin

The increased concentration of methemoglobin in this disorder occurs in the absence of any exogenous methemoglobin producing substances This serves to distinguish this syndrome from the acquired methemoglobinemias Since normal individuals have small but definite amounts of methemoglobin in their blood it is apparent that the formation of methemoglobin is a normal process Accordingly it must be assumed that there is a physiologic reducing system which can reconvert methemoglo

* The experimental work included in this report was completed while the author was a Research Fellow in the Department of Pediatrics Harvard Medical School and the Department of Medicine Children's Medical Center Boston Mass

bin to hemoglobin The existence of abnormal levels of methemoglobin indicates an imbalance between the oxidative and reductive processes

## HISTORICAL INTRODUCTION

The first reports of cyanosis due to an abnormal blood pigment (presumably methemoglobin) stressed a supposed relationship with gastrointestinal disturbances in the descriptive phrase enterogenous cyanosis [1] It was not until 1930 when Litarczek et al described an adult with cyanosis from early childhood [2] that the possibility of constitutional metabolic disorder was seriously entertained The familial nature of the syndrome was suggested by Hitzenberger [3] and later confirmed by Bensley et al in 1938 [4] The latter authors in their report surveyed the world literature on hereditary methemoglobinemia and found only four sporadic cases of the idiopathic (metabolic) type in addition to the two reports of familial cases then available Probably as a result of the decline in usage of methemoglobin producing drugs and of the increased interest in metabolic disease in general a remarkable number of new cases of the idiopathic type has been recorded since their survey More than 30 pedigrees are known which involve at least two affected members and a comparable number of isolated sporadic cases can be listed

The major efforts to elucidate the fundamental mechanism of this disease have been chiefly those of the English worker Gibson His studies of affected individuals from several families have demonstrated a defect in the enzyme system which is normally responsible for maintaining the hemoglobin in the reduced state [5] This defect appears to be transmitted recessively

Quite recently it has become apparent that Gibson s explanation is inapplicable to many of the reported cases The first note of discrepancy was introduced by the studies of Hörlein and Weber of a German family in which the disorder could be attributed to a dominantly transmitted abnormality in the hemoglobin itself [6] The dominant inheritance of congenital methemoglobinemia particularly in Greek populations has been stressed by Codounis [7] A number of North American families also demonstrating dominant transmission have now been studied and in all an abnormal hemoglobin has been found with properties quite sufficient to explain the methemoglobinemia (see below) [8] This group of abnormal hemoglobins is known as the Hb M type pigments after the example of Singer [9] who applied the designation *Hb M* to the hemoglobin discovered by Hörlein and Weber [6] These pigments are all characterized by the anomalous nature of their methemoglobin as indicated by the abnormal absorption spectrum of this derivative

From the foregoing it is evident that hereditary methemoglobinemia is not a single disease but rather a group of diseases On the basis of

current information three categories of differing etiology may be postulated (1) formation of an abnormal methemoglobin resistant to reduction, (2) a deficiency in the erythrocyte reducing system, with accumulation of "normal" methemoglobin, and (3) abnormal endogenous formation of oxidizing substances, with increased production of methemoglobin beyond the capacity of the system which normally maintains the hemoglobin in the reduced state

## CHEMISTRY OF METHEMOGLOBIN

### *"Normal" Methemoglobin*

Methemoglobin is the oxidized derivative of hemoglobin in which the iron of the four heme groups is changed from the usual ferrous to the ferric state As a consequence the iron is no longer able to complex with oxygen Methemoglobin is accordingly unable to serve as a respiratory pigment These statements apply with equal validity to the methemoglobin derived from all known hemoglobins including the abnormal human hemoglobins and the several molecular species that comprise "normal" hemoglobin [1]

As is true of heme pigments in general methemoglobin has a characteristic spectral absorption curve Employed in identification and measurement its use rests upon the implicit assumption that the methemoglobins prepared from different hemoglobins are identical or very nearly so in their spectral properties This assumption is quite valid for the various components of normal human hemoglobin and for most abnormal hemoglobins but the Hb M-type pigments must be excepted One should be aware that the absorption curve of methemoglobin changes with hydrogen ion concentration That observed at pH 7 0 and below corresponds to the acidic form of methemoglobin, and that at higher pH values to alkaline methemoglobin

Methemoglobin takes part in two major categories of reactions which are of particular interest here It may be reduced to hemoglobin which can then combine with oxygen (to form oxyhemoglobin) or it may complex directly with a number of substances especially nitrogenous compounds such as cyanide In either case the spectrum is altered to an absorption pattern characteristic of the resulting compound Under usual conditions these reactions affect equally all four heme groups of the molecule and are accompanied by a change in the total charge of the molecule In the case of cyanmethemoglobin the addition of the cyanide ion neutralizes one positive charge of each heme group (since one univalent anion combines with each ferric atom) and the net charge is equal to that of oxyhemoglobin [2]

[1] The heterogeneity of normal hemoglobin is discussed in detail in the section on the hemoglobinopathies (see Chap 34)

[2] Heme-linked ionizations are not considered in this discussion

*Gasometric Methods of Analysis*

The loss of oxygen-combining power may be used as a measure of the amount of methemoglobin by comparing the total oxygen capacity with that expected from the total amount of heme pigments present The latter is separately determined by estimation of the heme groups (as by iron analysis or as acid hematin) This method usually overestimates the concentration of methemoglobin since of necessity the result also includes other non-oxygen-combining pigments such as sulfhemoglobin This technique indicates that not more than 2 per cent of the hemoglobin of normal blood can be in the methemoglobin form [10]

*Spectroscopic Methods of Analysis*

In essence all spectroscopic methods assay the amount of methemoglobin by quantitating the spectral change when methemoglobin is chemically altered either by complex formation or by reduction The most commonly used technique is that of Evelyn and Malloy [11] The methemoglobin is reacted with cyanide and the change in light absorption at 632 mμ is measured This method is quite specific in the absence of the Hb M-type pigments The normal concentration of methemoglobin obtained with cyanide techniques is less than 2 per cent of the total pigment [12]

Since Hb M-type pigments in the methemoglobin form have abnormal absorption spectrums the usual quantitative spectroscopic tests give erroneous values for the degree of methemoglobinemia in Hb M disease With certain varieties of Hb M disease the circulating pigment does not react with cyanide and a negative result for methemoglobin is obtained in spite of the obvious cyanosis of the patient Qualitative spectroscopic tests which depend upon identification of the characteristic absorption band (at 632 mμ) will likewise not give the expected finding In many cases of Hb M disease no abnormal absorption band will be seen with the hand spectroscope

## HEREDITARY METHEMOGLOBINEMIA ASSOCIATED WITH THE SPECTROSCOPICALLY ABNORMAL METHEMOGLOBINS (THE Hb M DISEASES)

*Electrophoretic and Spectrochemical Behavior of Normal Hemolysates*

Since the isolation of the Hb M-type pigments has been accomplished only very recently the information concerning them is relatively limited and is confined largely to the electrophoretic and spectroscopic behavior of certain derivatives A few preliminary remarks concerning the electrophoretic behavior of hemolyzates prepared from the blood of normal individuals is a necessary introduction to this subject (a more extensive discussion will be found in the section on the hemoglobinopathies Chap

34) By utilizing various techniques, the hemoglobin of normal hemolyzates can be shown to contain a minimum of three distinct components (Hb $A_1$ Hb $A_2$ and Hb F) These probably differ considerably in their intimate chemical structure Under the conditions used in the study of the Hb M–type pigments (electrophoresis on starch block at pH 7 0) normal hemolyzate migrates as a single band toward the cathode Normal hemolyzate also migrates as a single band (toward the cathode) after oxidation to methemoglobin, although now the rate of migration is more rapid because of the increase in positive charge After neutralization of the charge by treatment with cyanide, the pigment (cyanmethemoglobin) migrates as a single band with essentially the same mobility as oxyhemoglobin

*Electrophoretic and Spectrochemical Behavior of the Hb M–Type Pigments*

In the blood from all individuals exhibiting methemoglobinemia of the dominantly transmitted type so far examined electrophoresis of

TABLE 33 1 SPECTROSCOPIC AND CHEMICAL PROPERTIES OF THE HB M-TYPE PIGMENTS (IN $M/15$ SODIUM PHOSPHATE BUFFER pH 6 5)

| *Pigment* | *Maxima in visible region mμ* | *Gross color of electrophoretic band* | *Cyanmethemoglobin spectrum*† |
|---|---|---|---|
| Methb A | 502 632 | Brown | Normal |
| Methb $M_B$ | 495 602 | Gray | Abnormal |
| Methb $M_S$ | 492 602 | Green | Normal |
| Methb $M_M$ | 500 602 | Gray green | Normal |

Hb M Boston type (Hb $M_B$) was isolated from the family reported by Gerald et al [13] Hb M Saskatoon type (Hb $M_S$) from that of Baltzan and Sugarman [14] and Hb M Minneapolis type (Hb $M_M$) from that of Pisciotta and Hinz [15]

† Those pigments with an abnormal cyanmethemoglobin spectrum are not associated with increased concentration of methemoglobin in the patient when the methemoglobin is determined by its reaction with cyanide

hemolyzates under appropriate conditions has disclosed an abnormal pigment These pigments collectively known as the Hb M–type pigments are anomalous in that the relative electrophoretic mobilities of the oxyhemoglobin, methemoglobin and cyanmethemoglobin derivatives differ from that predicted by experience with normal hemoglobin (Fig 33-1) The untreated hemolyzate migrates as a single band After conversion of all the pigment to methemoglobin by treatment with ferricyanide two distinct bands are observed After conversion to cyanmethemoglobin a single band is again observed which has a mobility comparable to that of the initial hemolyzate These findings are incompatible with the charge differences among oxyhemoglobin methemoglobin and cyanmethemo-

globin derivatives as previously described, and are best explained by assuming that the increase in positive charge of the methemoglobin, over that present in the oxyhemoglobin and cyanmethemoglobin forms is less for the Hb M-type pigments than for normal hemoglobin Very little or no charge difference apparently exists between the normal and the

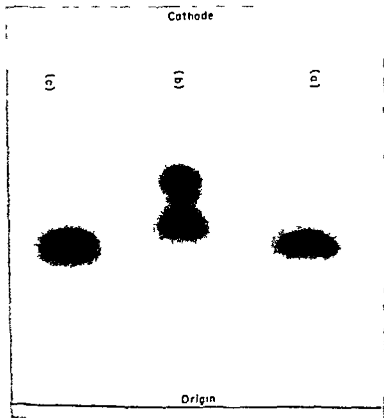



Fig 33 1 Electrophoretic behavior on starch block of a hemolyzate containing both Hb M and Hb A (a) Untreated hemolyzate (b) after oxidation to methemoglobin (c) after conversion to cyanmethemoglobin (Electrophoresis in sodium phosphate buffer pH 7 0 ionic strength 0 10)

abnormal fractions of the hemolyzate when present as oxyhemoglobin or cyanmethemoglobin a significant difference appears only when the methemoglobins are compared

The electrophoretically purified Hb M-type pigments have been studied spectroscopically and are most unusual [8] The absorption maxima of the acidic methemoglobin form of three different Hb M-type pigments are given in Table 33-1 These are designated Hb $M_B$ Hb $M_S$

instance the usual methemoglobin band could not be demonstrated in a patient who was intensely cyanotic [20] Finally, the pedigree of sulfhemoglobinemia described by Miller [26] and that of idiopathic cyanosis by Lutembacher [27] cannot be distinguished from instances of Hb $M_B$ disease by the tests reported

*Erythrocyte Metabolism in the Hb M Diseases*

The limited studies performed to date have failed to demonstrate any abnormality of the erythrocyte enzyme systems The rate of reduction of methemoglobin with cells treated in vitro with oxidizing agents has not differed significantly from that observed with normal red cells even under the conditions which successfully demonstrate the defect in the enzymatic variety of hereditary methemoglobinemia [28] Pisciotta et al [15] observed in one patient[4] that the concentration of methemoglobin in freshly shed blood did not change after incubation of the cells with fresh normal plasma or with glucose Also if the concentration of methemoglobin in the cells was first increased to 90 per cent (from the in vivo level of 30 per cent) by treatment of the cells with nitrate incubation with fresh normal plasma resulted in a decrease of methemoglobin to the original 30 per cent value in a few hours This result is in accord with a normal enzyme system which can convert normal methemoglobin to the reduced state but which is less able to cope with abnormally reactive oxidized hemes of the Hb M-type pigments

The life span of the erythrocyte as determined by reticulocyte count and serum bilirubin is normal in the Hb M diseases The one reported exception to this was in a patient possessing both a Hb M-type pigment and an additional unidentified (Hb C like) hemoglobin [21]

*Clinical Physiology*

On the basis of the proposed theory it is reasonable to expect that the methemoglobin formed in vivo in Hb M diseases would be derived almost solely from abnormally reactive hemes of the Hb M-type pigments The failure to detect abnormal blood concentrations of methemoglobin by the Evelyn and Malloy test in two families is consistent with this [18 21] As a result there is an upper limit to the impairment in oxygen combining power which on this basis should not exceed one-half the concentration of the Hb M-type pigment The abnormal reactivity is also responsible for the resistance to ascorbic acid and methylene blue therapy seen in some cases of Hb M disease [13 21]

[4] A Hb M-type pigment (Hb $M_M$) has since been isolated by the author from the blood of this patient samples of which were generously made available by Dr Pisciotta

*Genetics*

Since the abnormality in the Hb M-type pigments is limited to the globin of the molecule the e hemoglobins may be classed with the more common abnormal hemoglobins (see Chap 34) Like them the Hb M-type pigments have been transmitted as a codominant character involving both sexes in all the pedigrees adequately studied No abnormality has been commonly associated with the abnormal hemoglobin except tho e secondary to its pre ence All reported cases have come from European and Mediterranean populations

## HEREDITARY METHEMOGLOBINEMIA ASSOCIATED WITH A DEFICIENCY OF THE METHEMOGLOBIN REDUCING SYSTEM

*Methemoglobin Reduction in the Normal Human Erythrocyte*

It is as umed that the small but definite amount of methemoglobin in normal blood represents an equilibrium between the proces es causing methemoglobin formation and those accomplishing its removal Except for the effect of oxygen little information is available concerning the factors responsible for the normal formation of methemoglobin hence the following remarks will be confined to the methemoglobin reducing process Since there is a significant interspecies variation in these reactions the di cussion will be confined to results obtained in studies of human erythrocytes

The reduction of methemoglobin in the normal human red cell proceeds slowly in a saline su pension but is increased by the addition of glucose to the medium and nearly equally so by the addition of lactate [29] With either ubstrate the reduction is accompanied by the formation of an equivalent amount of pyruvate [29] ( ee Fig 33 2 for a conden ed outline of the glycolytic pathway) Methemoglobin reduction is probably linked to glycolysis through reduced dipho phopyridine nucleotide (DPNH) [29] Evidence for this is the inhibitory action of iodoacetate on methemoglobin reduction when gluc se is the ub trate and its lack of effect when the gluco e is replaced by lact ate ( ee Fig 33 2) In keeping with this scheme is the failure of fluoride to affect reduction in the presence of glucose [29]

Certain autoxidizable dyes uch as methylene blue (MB) are capable of accelerating the reduction of methemoglobin in the presence of the proper sub trate When MB is added al ng with glucose a tenfold incre e is obtained The amount of pyruvate (or of phosphoglycerate when fluoride is present) formed under these conditions is con iderably less than that expected from the amount of methemoglobin reduced in contra t to the results obtained without MB The MB thus appears to

activate a metabolic pathway which is not operative in its absence [29] From studies of normal and hereditary methemoglobinemic erythrocytes, Gibson concluded that this pathway was the hexose monophosphate shunt [29] the initial and pertinent steps of which are given in Fig 33 3

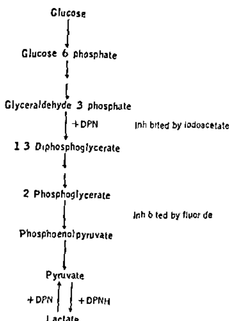



Fig 33 Condensed outline of the glycolytic pathway [30] The omission of intermediate reactions is indicated by a double arrow

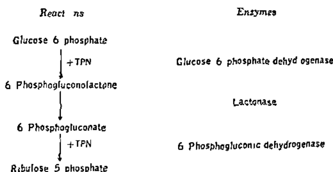



Fig 33-3 Initial reactions of the hexose monophosphate shunt [31]

It should be noted that TPNH rather than DPNH is produced by this reaction Indirect evidence supporting Gibson s contention is now available from the studies of cells deficient in glucose 6 phosphate dehydrogenase (G 6 P D) Red cells deficient in G 6-P D activity are concomitantly defective in reducing methemoglobin in the presence of glucose plus MB [32 33]

The majority of investigators though by no means all believe that the reaction between methemoglobin and reduced pyridine nucleotides is an enzymatically controlled step Kiese obtained direct evidence in favor of this by preparing an extract from horse erythrocytes which was capable of reducing methemoglobin in the presence of TPNH [34] His term for the enzyme *methemoglobin reductase* has since been adopted in the current literature for the human counterpart Gibson assumed the existence of such an enzyme but postulated that two comparable systems were present one active toward TPNH and the other toward DPNH [29] Recently Huennekens et al isolated an enzyme (which they called methemoglobin reductase) from human erythrocytes which catalyzed the reaction of methemoglobin with TPNH [35] Unexpectedly this preparation had a significant though lesser (one-fifth as great) activity with DPNH

A preliminary account is now available of an investigation furnishing direct evidence for the presence in normal human erythrocytes of an enzyme capable of reducing methemoglobin which has a DPNH preference [36] In this and in other properties it differs significantly from the methemoglobin reductase of Huennekens et al Its probable importance in the reduction of methemoglobin is indicated by the observation that this particular enzyme is deficient in the erythrocytes from the few patients with hereditary methemoglobinemia (of the recessively transmitted type) so far studied

*Methemoglobin Reduction in Erythrocytes from Patients with Enzymatic Hereditary Methemoglobinemia*

It has long been suspected that the abnormality in hereditary methemoglobinemia is an intrinsic disorder of the erythrocyte Lian et al found that methemoglobin has little or no tendency in vitro to disappear spontaneously from hereditary methemoglobinemic cells [37] in contrast to the rapid reduction observed with cells from toxic methemoglobinemia A more dramatic and elaborate confirmation of this clinical conclusion was obtained by Eder et al who transfused cells from a congenitally methemoglobinemic individual into a patient with aplastic anemia and demonstrated the persistence of methemoglobin within the erythrocytes despite their being in a foreign circulation [38]

Incubation of erythrocytes from patients with hereditary methemoglobinemia of the enzymatic type in saline containing glucose is not associated with the rapid reduction of the methemoglobin which is seen with normal cells in which methemoglobin has been produced by chemical treatment This is observed in spite of apparent adequacy of the glycolytic enzyme systems in such cells [29] Preservation of glycolysis is inferred from the rapid reduction of pyruvate to lactate in the presence of glucose plus fluoride as in normal cells However in the presence of both MB and glucose normal and hereditary methemoglobinemia cells are equally

activate a metabolic pathway which is not operative in its absence [29] From studies of normal and hereditary methemoglobinemic erythrocytes Gibson concluded that this pathway was the hexose monophosphate shunt [29], the initial and pertinent steps of which are given in Fig 33 3

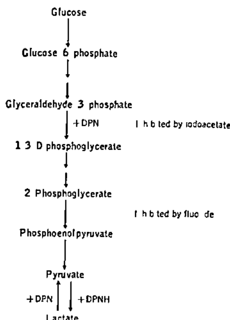



Fig 33 2 Condensed outline of the glycolytic pathway [30] The omission of intermediate reactions is indicated by a double arrow

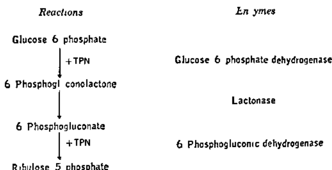



Fig 33-3 Initial reactions of the hexose monophosphate shunt [31]

It should be noted that TPNH rather than DPNH, is produced by this reaction Indirect evidence supporting Gibson s contention is now available from the studies of cells deficient in glucose 6 phosphate dehydrogenase (G 6 PD) Red cells deficient in G 6 PD activity are concomitantly defective in reducing methemoglobin in the presence of glucose plus MB [32 33]

definite shift to the left of the curve was observed [3 42–44] and in one instance a slight shift [2] but in two no alteration could be demonstrated [38 45] Gibson has emphasized the significance of this discrepancy and has suggested that absence of the alteration in the oxygen dissociation curve implies an uneven distribution of methemoglobin i e some erythrocytes contain only methemoglobin and the remainder only reduced (ferro ) hemoglobin [5] It is of course possible that these are two groups of cases which differ basically in their etiology

*Clinical Physiology and Therapy*

The defect in oxygen carrying capacity of the blood of the e patients has not been sufficient to cause symptoms referable to oxygen deficiency except in a few cases nor has there been a direct effect upon the survival of the red cell

The therapy of this disease is based upon the promotion of in vivo reduction of the methemoglobin by administration of MB or ascorbic acid Although ascorbic acid is nearly as effective as MB its mode of action is still but poorly understood MB administered intravenously causes an abrupt fall of the methemoglobin concentration to near normal values while ascorbic acid administration is associated with a gradual decline to a steady level which is still somewhat above normal [45] Alleviation of the cyanosis with either of these agents is obtained only so long as therapy is continued

The intensity of cyanosis varies with the diet and with the season which may possibly be a reflection of ascorbic acid intake The onset of cyanosis is variable In a few cases it has been observed immediately or a few hours after birth [46] In others it has not been noticed until adolescence [4] Whether this is related to diet or to exposure to methemoglobin promoting agents is not known

Although the appearance of cyanosis in early infancy suggests the very real possibility of hypoxic brain damage this complication has been reported relatively infrequently [3 46–49] The occurrence of mental deficiency in all the methemoglobinemic children (total of six affected) of two pedigrees [47, 48] suggests a unique difference in the etiology of their methemoglobinemia

*Genetics*

Until recently information on the inheritance of hereditary methemoglobinemia was confused by pedigrees documenting both recessive and dominant transmission Some of the families with dominantly inherited cyanosis are now known to be affected with one of the Hb M diseases and in many of the remaining families indirect evidence suggests that they also belong to this same category On the basis of pre ent information it seems reasonable to anticipate that the two inheritance patterns will

able to reduce methemoglobin It was this contrasting behavior which led Gibson to postulate that MB activates an otherwise inactive reduction pathway [29] On the assumption that reduction of methemoglobin (in the absence of MB) by DPNH is controlled by a specific DPNH dependent enzyme Gibson considered that a deficiency of this enzyme was the basis of the defect in hereditary methemoglobinemia [29] Gibson developed an assay method supposedly specific for this erythrocytic enzyme and was able to demonstrate a marked deficiency in five methemoglobinemic patients and a slight deficiency in two others [29 39]

As previously mentioned, further evidence is now available to corroborate Gibson s hypothesis that methemoglobin reduction is normally controlled by a DPNH-dependent enzyme [36] The direct demonstration of this enzymatic activity was made possible by the development of an improved assay system Scott and Griffith have shown that the reaction between 2,6-dichlorobenzenone indophenol and DPNH is catalyzed by an enzyme present in the hemolyzates from normal individuals The hemolyzates from patients with hereditary methemoglobinemia however failed to exhibit such activity [40]

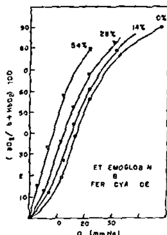



Fig 33-4 The effect of methemoglobin as formed by ferricyanide on the oxygen dissociation curve of human hemoglobin 0 6 M phosphate buffer pH 7 4 With increasing methemoglobin the dissociation curve shifts to the left [41]

### *The Oxygen Dissociation Curve in Hereditary Methemoglobinemia*

Methemoglobin is unable to bind oxygen because of the altered state of the iron In addition there is an indirect effect resulting from the interaction of the four heme groups in the hemoglobin molecule Oxidation of one of the heme groups in a given molecule affects the tenacity of the binding with oxygen of the remaining unoxidized groups This is one manifestation of a more general phenomenon known as heme-heme interaction This is most clearly demonstrated by plotting oxygen saturation of the hemoglobin against oxygen pressure of the gaseous phase the oxygen dissociation curve The heme heme interaction theory predicts that following partial oxidation of the hemes a change in shape of the curve and a shift to the left (greater saturation at lower oxygen tension) will occur This prediction was confirmed by the experimental findings of Darling and Roughton with hemoglobin after partial oxidation in vitro [41] (Fig 33-4)

The oxygen dissociation curve in hereditary methemoglobinemia has been determined by seven different investigators In four instances a

(b) a deficiency in the erythrocyte enzyme system which maintains hemoglobin in the unoxidized state and (c) abnormal endogenous formation of substances capable of oxidizing hemoglobin to methemoglobin Although only a single possible case is available to illustrate the last category the first two pathogenetic mechanisms appear to be of nearly equal importance in the causation of hereditary methemoglobinemia

3 The frequency of abnormal methemoglobins resistant to reduction has only recently been realized These methemoglobins which are the oxidation products of a group of different hemoglobins categorically designated the Hb M-type pigments are unique in their electrophoretic and spectrochemical behavior Their spectral absorption curves differ from that of methemoglobin prepared from normal human blood and hence from that of any other vertebrate methemoglobin Consequently the usual spectroscopic and chemical tests for methemoglobin may not be applied to blood containing a Hb M-type pigment without risking quantitative or even qualitative error

Since the molecular abnormality of the Hb M-type pigments lies in the globin these may properly be considered as further examples of the abnormal hemoglobins As might be expected in pedigrees exhibiting this condition the cyanosis is transmitted as a dominant character

4 The second major pathogenetic category of hereditary methemoglobinemia derives from a deficient intraerythrocytic enzyme system for reducing methemoglobin Present evidence favors the theory that the disease arises from a deficiency of a diphosphopyridine nucleotide—dependent enzyme which is normally responsible for reducing methemoglobin

5 Alternatively hereditary methemoglobinemia of the enzyme deficiency type may be a heterogeneous group of conditions Of particular interest in this regard is the seemingly inextricable association of hereditary methemoglobinemia and mental defect in some families and the unexpected finding of oxygen dissociation curves without the shift to the left obtained in others

6 The two main categories of hereditary methemoglobinemia differ not only in spectroscopic findings but also on genetic grounds It seems probable that the pedigrees with dominant transmission of cyanosis represent instances due to a Hb M type pigment and that the recessive variety is due to erythrocytic enzyme deficiency

## BIBLIOGRAPHY


1 van den Bergh A and Grutterink A Enterogene Cyanose Berl klin Wchnschr 43 7 1906

2 Litarczek G Aubert H Cosmulescu I Comanesco V and Litarczek S Sur un cas de cyanose auto toxique par méthémoglobinémie intra-globulaire Sang 4 188 1930

divide the cases into Hb M disease (dominant) and the enzyme deficiency variety (recessive)

When a rare disease is recessively inherited the proportion of pedigrees derived from consanguineous matings is usually appreciable Surprisingly, only a single instance of consanguinity has been noted among the pedigrees of hereditary methemoglobinemia of the recessive type [49]

Like the Hb M diseases the enzyme deficiency variety has been described only in populations with a light complexion This may reflect the difficulty of detecting cyanosis in a dark skinned person Both European and non European peoples [40, 50] have been affected No predilection for either sex has been noted

## CONGENITAL METHEMOGLOBINEMIA DUE TO AN EXCESS OF ENDOGENOUS OXIDIZING SUBSTANCES

At present this category is largely hypothetical One case has been reported which may fall within it Fishberg has described a patient with idiopathic methemoglobinemia and severe cyanosis (no clinical details were given) who excreted large amounts of benzoquinoneacetic acid in the urine even *while on a normal diet* [51] Methemoglobin concentrations as high as 20 per cent were observed during the period of benzoquinoneacetic acid excretion and the actual blood concentration of methemoglobin varied roughly with the output of this substance Ascorbic acid administration rapidly abolished both the excretion of benzoquinoneacetic acid and the methemoglobinemia It was concluded by Fishberg that the methemoglobinemia was the direct result of the oxidation of the hemoglobin by benzoquinoneacetic acid Although the data are not adequate to determine whether the presence of the latter resulted from a metabolic disorder or from a transient ascorbic acid deficiency the frequency of ascorbic acid deficiency without concomitant cyanosis suggests that a metabolic error was present in Fishberg s case

## SUMMARY

1 Hereditary methemoglobinemia is characterized by chronic cyanosis associated with increased amounts of intraerythrocytic methemoglobin the oxidation product of hemoglobin Since small amounts of methemoglobin may be identified in normal blood it is believed that an equilibrium exists between the processes responsible for oxidation of the hemoglobin to methemoglobin and those which facilitate the reverse reaction Hereditary methemoglobinemia is the result of a hereditary imbalance in these two competing processes

2 At present three different pathogenetic mechanisms appear possible (*a*) the formation of an abnormal methemoglobin resistant to reduction,

31 Horecker B and Mehler A Carbohydrate metabolism Ann Rev Biochem 24 207 1955
32 Dawson J Thayer W and Desforges J Acute hemolytic anemia in the newborn infant due to naphthalene poisoning report of two cases with investigations into the mechanism of the disease Blood 13 1113 1958
33 Motulsky A Personal communication
34 Kiese M Die Reduktion des Hämoglobins Biochem Ztschr 316 264 1944
35 Huennekens F Caffrey R Basford R and Gabrio B Erythrocyte metabolism IV Isolation and properties of methemoglobin reductase J Biol Chem 227 261 1957
36 Scott E and Griffith I The enzymic defect of hereditary methemoglobinemia diaphorase Biochim et biophys acta 34 584 1959
37 Lian C Frumusan P and Sassier M Méthémoglobinémie congénitale et familiale Action favorable de l acide ascorbique Bull et mém Soc méd hôp Paris 55 1194 1939
38 Eder H Finch C and McKee R Congenital methemoglobinemia A clinical and biochemical study of a case J Clin Invest 28 265 1949
39 Breakey V Gibson Q and Harrison D Familial idiopathic methaemoglobinaemia Lancet 1 935 1951
40 Scott E and Hoskins D Hereditary methemoglobinemia in Alaskan Eskimo and Indians Blood 13 795 1958
41 Darling R and Roughton F The effect of methemoglobin on the equilibrium between oxygen and hemoglobin Am J Physiol 137 56 1942
42 Gibson Q and Harrison D Familial idiopathic methaemoglobinaemia Lancet 2 941 1947
43 Darling R cited by Eder et al (ref 38)
44 Barkie A and Valtis D Gas transport function of the blood in congenital familial methaemoglobinaemia Brit M J 2 73 1954
45 Waisman H Bain J Richmond J and Munsey F Laboratory and clinical studies in congenital methemoglobinemia J Lab & Clin Med 10 293 1952
46 Dine M Congenital methemoglobinemia in the newborn period A M A J Dis Child 92 15 1956
47 Worster Drought C White J and Sargent F Familial idiopathic methaemoglobinaemia Brit M J 2 114 1953
48 Kay W and MacMahon J Idiopathic familial methaemoglobinaemia (two cases) Proc Roy Soc Med 46 717 1953
49 Gibson R Familial idiopathic methaemoglobinaemia associated with oligophrenia Am J Ment Deficiency 61 207 1956
50 Chang H and Wu S Congenital methemoglobinemia Report of a case Chinese M J 72 153 1954
51 Fishberg E Excretion of benzoquinoneacetic acid in hypovitaminosis C J Biol Chem 172 155 1948

3 Hitzenberger K Autotoxische Zyanose (Intraglobuläre Methämoglobinämie) Wien Arch inn Med 23 85 1932
4 Bensley E Rhea L and Mills E Familial idiopathic methaemoglobinaemia Quart J Med 7, (N S) 325 1938
5 Gibson Q Methaemoglobin and sulfhaemoglobin Biochem Soc Symposia (Cambridge Engl) 12 55 1954
6 Horlein H and Weber G Über chronische familiäre Methämoglobinämie und eine neue Modifikation des Methämoglobins Deutsche med Wchnschr 73, 476 1948
7 Codounis A A genetic study on the hereditary methaemoglobinaemic cyanosis (H M C) Acta genet 7, 131 1957
8 Gerald P and George I Second spectroscopically abnormal methemoglobin associated with hereditary cyanosis Science 129 393 1959
9 Singer K Hereditary hemolytic disorders associated with abnormal hemoglobins Am J Med 18 633 1955
10 Gibson Q and Harrison D Blood haemoglobin the relationship between oxygen capacity and iron content of blood in men and women Biochem J 40 247 1946
11 Evelyn K and Malloy H Microdetermination of oxyhemoglobin methemoglobin and sulfhemoglobin in a single sample of blood J Biol Chem 126 655 1938
12 van Slyke D Hiller A Weisiger J and Cruz W Determination of carbon monoxide in blood and of total and active hemoglobin by carbon monoxide capacity Inactive hemoglobin and methemoglobin contents of normal human blood J Biol Chem 166 121 1946
13 Gerald I Cook C and Diamond L Hemoglobin M Science 126 300 1957
14 Baltzan D and Sugarman H Hereditary cyanosis Canad M A J 62 348 1950
15 Fisciotta A and Hinz J Hereditary methemoglobinemia Clin Res Proc 5, 291 1957
16 Gerald I The electrophoretic and spectroscopic characterization of Hgb M Blood 13 936 1958
17 George P Personal communication
18 Horlein H and Weber G Chronische familiare Methämoglobinämie Ztschr ges inn Med 6 197 1951
19 Ingram V Gene mutations in human haemoglobin the chemical difference between normal and sickle cell haemoglobin Nature London 180 3 6 1957
20 Hansen H Jagenberg R and Reinand T A new abnormal Fe hemochromogen as a cause of hereditary cyanosis Acta chem scandinav 9 1035 1955
21 Fisciotta A Ebbe S and Hinz J Hemoglobin M disease J Lab & Clin Med 50 939 1957
22 Heck W and Wolf H Angeborene Herzfehler mit Cyanose durch pathologischen Blutfarbstoff (Hämoglobin M) Ann paediat 190 135 1958
23 Rossi Fanelli A Antonini E and Mondovi B Ferrihemoglobin reduction in normal and methemoglobinemic subjects Clin chim acta 2 476 1957
24 Codounis A Hereditary methaemoglobinaemic cyanosis Brit M J 2 368 1952
25 Codounis A Le syndrome clinico-biologique de la cyanose méthémoglobinémique héréditaire (C M H) entité morbide au globule rouge maladie autonome bien définie Semaine hôp Paris 28 3876 1952
26 Miller A Congenital sulfhemoglobinemia J Pediat 51 233 1957
27 Lutembacher R Cyanose congénitale héréditaire et familiale Presse méd 57 276 1949
28 Kiese M Kurz H and Schneider C Chronische Hamoglobinamie durch pathologischen Blutfarbstoff Klin Wchnschr 34 957 1956
29 Gibson Q The reduction of methaemoglobin in red blood cells and studies on the cause of idiopathic methaemoglobinaemia Biochem J 42 13 1948
30 Baldwin E *Dynamic Aspects of Biochemistry* 3d ed pp 414 and 415 Cambridge University Press Cambridge 1957

## ADULT AND FETAL HEMOGLOBIN

About one-hundred years ago von Korber [1] noticed that the blood of the adult is more easily denatured by alkali than that from the placenta He concluded that there are two human hemoglobins adult and fetal These are now designated A and F Hemoglobin F is present in infants at birth when it varies in concentration from approximately 60 to 90 per cent It then gradually disappears from the circulation and by the age of 4 months only traces persist ✓

Jonxis [2] has pointed out that the presence of Hb F in the blood of normal children over 3 months old may be explained only by the synthesis of this pigment after birth Formation of Hb F may persist in pathologic states which begin at an age when Hb F is still being produced and if the cause of the infantile disorder usually an anemia is not removed Hb F may still be found in adult life This was first observed in thalassemia [3-6] but was later also found in sickle cell anemia [7] and other hemoglobinopathies The phenomenon is not specifically associated with this group of disorders but can be observed in many other syndromes such as spherocytic jaundice leukemia and nutritional anemias

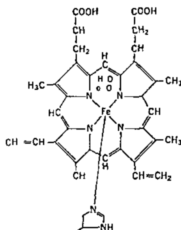



FIG 34 1 Heme one of the four identical prosthetic groups attached to one molecule of globin

The numerous differences between adult and fetal hemoglobins have been summarized by White and Beaven [8] and by Betke [9] These include differences in solubility ultraviolet spectrum and resistance to alkali denaturation There may be differences also for oxygen affinity and in antigenic activity Hemoglobin F is inherited independently of all the adult hemoglobin variants These and other properties listed below divide it sharply from Hb A and its variants

*Alkali Denaturation* At pH 12 8 (0 04 N alkali) and at room temperature the half reaction time of denaturation ($t_{1/2}$) for Hb A is 10 sec and for Hb F is 1 000 sec

*Ultraviolet Spectrum* The tryptophan fine-spectrum band for Hb A is 291 0 mμ and for Hb F is 289 8 mμ

*Electrophoresis* The isoelectric point of Hb A is 6 87 and of Hb F is 6 98 Upon boundary electrophoresis at pH 6 5 A and F separate On paper electrophoresis at pH 8 6 A and F do not separate but when present in a mixture F causes slowing of the A band Upon starch gel electrophoresis at pH 8 6 Hb F separates as a distinct band behind Hb A With agar electro-

## Chapter 34

# The Hemoglobinopathies and Thalassemia

---

Hermann Lehmann and J. A. M. Ager

The observations of Pauling and his collaborators on the electrophoretic characteristics of human hemoglobins first published in 1949 form a landmark in biochemical genetics. Their observations and many which have followed have firmly established the existence of various species of human hemoglobin and have shown that a subject may have various combinations of these species circulating in his blood at the same time. The inheritance of the hemoglobins follows strict genetic patterns and certain dyscrasias of the blood may quite precisely reflect the genetic pattern of the constituent hemoglobins.

This chapter will include a review of present information on the chemical nature of the hemoglobins and the genetics of their inheritance and will attempt to classify the variant hemoglobins. In addition the diseases arising from an endowment of abnormal hemoglobin will be described and interpreted, and present information regarding the nature of thalassemia discussed.

## HUMAN HEMOGLOBIN

Hemoglobin is the principal protein of man concerned with oxygen transport. Each molecule of hemoglobin consists of one molecule of globin to which are attached four molecules of ferroprotoporphyrin IX (Fig. 34.1). This prosthetic group is common to groups of compounds concerned in redox processes in both the plant and animal kingdoms from the peroxidase of horse radish to the cytochromes of man. The protein part of the hemoglobin molecule shows a wide variation of molecular weight in the lower animals but in the vertebrates the molecular weight is relatively constant at about 68 000 (66 700 for man).

component, Hb $A_1$, and a minor component amounting to about 3 per cent Hb $A_2$ [12]

Hemoglobin $A_2$ is a definite separate entity It is noteworthy that it is found in the absence of Hb $A_1$ in sickle cell homozygotes Also like Hb F it is increased in thalassemia in which Hb $A_1$ formation is decreased Occasionally Hb $A_2$ separates into two fractions on electrophoresis $A_2$ and $A_2'$ [13 14] (Fig 34 2) The trait $A_2 + A_2$ segregates as a simple Mendelian alternative to single $A_2$ Matings of persons with $A_2$ and $A_2A_2$ produce both type in equal proportions in the offspring

A minute fraction on the leading edge of the $A_1$ band may also appear upon electrophoresis of normal hemoglobin This small pointed area of hemoglobin has been designated Hb $A_3$ Its significance is not yet clear nor is that of a small fraction of hemoglobin which may also be seen in front of the A band on chromatography at pH 6 As will be discussed later the two $\alpha$ and two $\beta$ polypeptide chains which together form the globin molecule differ in electrophoretic and chromatographic properties Dissociation and recombination of these chains might take place in unequal proportions particularly at slightly acid pH This could cause the appearance of mall fractions differing from $A_1$ on electrophoresis and chromatography

## DIFFERENT ADULT HEMOGLOBINS

Until a few years ago interest in adult hemoglobin was centered on its prosthetic group the heme Carboxyhemoglobinemia sulfhemoglobinemia and methemoglobinemia were of clinical as well as of theoretic importance and it was known that methemoglobinemia could be either acquired or inherited The inherited type was attributed to abnormalities in reducing systems which normally counteract the tendency toward methemoglobin formation in the red cells (cf Chap 33) Hörlein and Weber [15] studied a family in which the condition appeared to be associated with the hemoglobin molecule for when globin and heme of the affected members of this family were separated and combined with normal heme and globin respectively the abnormality went with the globin part of the respiratory pigment Watson and her collaborators [16] had noted that the sickling phenomenon was only slight in infants and became more pronounced later when the fetal variant had been replaced by adult hemoglobin It was the discovery of Pauling and his collaborators [17] of a second adult human hemoglobin in sickle-cell blood that became a milestone along the road of twentieth century investigative medicine From that discovery has flowed an abundance of new light on the chemistry of human proteins the physiology of hemoglobin and the nature of numerous disease states of the blood Few single papers have been the beginning of so many lines of investigation many of them of far reaching consequence

chromotography' at pH 6 2 Hb F separates as a distinct band in front of Hb A

*Chromatography* Hemoglobin F separates as a distinct band in front of Hb A on Amberlite IRC 50 at pH 6 or on carboxymethylcellulose

*Solubility* Reduced Hb F is more soluble in phosphate buffer than is reduced Hb A

*Amino Acid Composition* There are numerous differences The most notable is in isoleucine content which is absent from pure Hb A and is 1 85 per cent in Hb F The four terminal residues in Hb A are valyl only but in Hb F there are two valyl and two glycyl residues

*Immunology* Antisera have been prepared from chickens rabbits and other species which precipitate specifically Hb A and F respectively Agglutinating serums have been prepared from rabbits which act on the red cells of the newborn but not on those of the adult except when the adult blood contains Hb F

*Oxygen Dissociation* The oxygen affinity of cord blood is greater than that of adult blood The difference disappears after dialysis against phosphate buffer

HETEROGENEITY OF NORMAL ADULT HEMOGLOBIN

Hemoglobin A is not a homogeneous protein Derrien [10] has summarized his own experiences and that of other workers with fractional

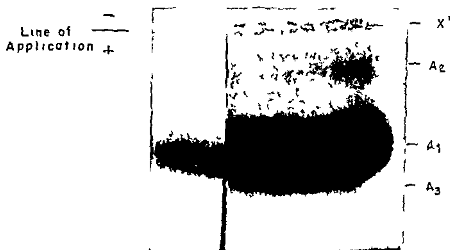



Fig 34 2 The heterogeneity of hemoglobin When normal adult hemoglobin is examined by paper electrophoresis at pH 8 6 a single fraction is observed (see left unstained strip) On staining several additional minor fractions appear These are Hb A and $A_3$ and a nonhemoglobin component X

"salting out" of Hb A He found six different components Itano [11] considers these results due to the transition of soluble protein into the solid phase where the crystal forms differ at different salt concentrations There can be no doubt that adult hemoglobin consists of at least two fractions which can easily be separated by electrophoresis a major

gested in 1947 [26] that there might be an alternative hypothesis similar to that which had differentiated between severe and mild thalassemia in terms of their being homozygous and heterozygous states In 1949 the same theory together with supporting evidence was put forward by Beet [27] and Neel presented conclusive proof based on numerous family studies [28]

The discovery of the sickle cell hemoglobin correlated all the data ✓ that had been accumulating Sickling was due to an abnormal hemoglobin

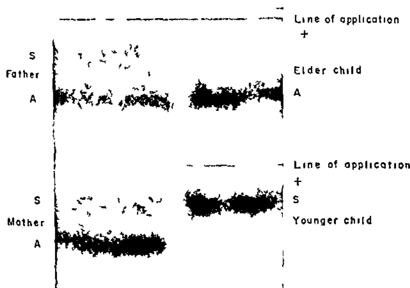



Fig 34-4 The electrophoretic pattern of a sample of blood obtained from two children of parents each of whom has a sickle-cell trait carrier Run at pH 8 6 On the left above a sample from the father below one from the mother On the right are samples from the elder child and the younger child

and whereas in the heterozygous carriers of the sickle-cell trait both Hb A and Hb S were found no A was present in patients with sickle cell anemia who were homozygous for the abnormal gene Thus the gene for Hb A found expression in the phenotype in the presence of another for Hb S (Fig 34-4) Another hemoglobin besides S was found in small amounts in the blood of patients homozygous for sickling but this was not Hb A It was later shown to be Hb F persisting in these patients beyond the age of infancy [7]

Almost all the findings in sickle-cell disease can be explained by the physical properties of reduced Hb S and particularly by its low solubility Harris [ J] found that the deoxygenation of concentrated solutions of Hb S results in a semi olid gel Under the microscope tactoids 1 to 15 $\mu$ long may be observed which are remarkably similar in shape to sickled

*Sickle cell Hemoglobin (Hemoglobin S)*

In 1910 Herrick [18] examined the blood of a severely anemic West Indian Negro student residing in Chicago and found it to contain "peculiar elongated and sickle-shaped red corpuscles" It was soon found that some Negroes had erythrocytes which appeared to be perfectly normal in vivo, but which could be induced to take on the sickle shape in vitro Hahn and Gillespie [19] reported that sickling occurs only with low oxygen tensions and that sickled cells can revert to the normal shape



Fig 34 3 Sickle cells from the peripheral blood as they appear under low oxygen tension

when they are exposed to sufficient oxygen (Fig 34 3) Diggs et al [20] distinguished between those individuals in whom the sickling of the red cells was associated with a profound disease sickle-cell anemia and others in whom the abnormality was a harmless trait Sherman [21] showed that the two conditions also differ in their tendency to sickle on deoxygenation Later it was seen that the first type of cell has a shorter life span than normal cells and that the trait cells have a normal survival time even when transfused into patients with sickle cell anemia [22, 23]

The sickling phenomenon is familial and the mode of inheritance is that of a single Mendelian dominant [24 25] Expression of a gene in this way in the heterozygote is now often referred to as codominance At first it was generally assumed that the same gene produces in some persons an asymptomatic condition the sickling trait and in others is responsible for the severe form with anemia and other changes Neel sug

of two other abnormal hemoglobins When genes for Hb A and Hb S are inherited together both hemoglobins are incorporated into the same cells This can be concluded because in sickle cell preparations from trait carriers all cells take on the abnormal shape However the proportion of



Fig 34 5 X ray appearance of the skull of an adolescent patient with sickle-cell anemia Note the thickened diploë and virtual absence of an outer table There appears to be a ring infarct in the frontal region (*By permission of Dr W P Cockshutt University College Hospital Ibadan Nigeria*)

the two hemoglobins is unequal only 20 to 40 per cent is Hb S Accordingly the manufacture of S must proceed more slowly That Hb A production prevails over that of S explains why sickle-cell heterozygotes carry the abnormal hemoglobin but do not suffer from the anemia

*Hemoglobin C*

The observation of atypical cases of sickle-cell disease with failure to find sickling in one of the two parents led to the discovery by Neel and his colleagues of a new second abnormal hemoglobin in the nonsickling parent and to the demonstration of the same pigment in the children with the intermediate type of sickle-cell disease [37 38] In these patients hemoglobin S amounts to more than half the total hemoglobin the con

cells On reoxygenation the tactoids disappear but they form again when the oxygen is removed Perutz and his collaborators [30, 31] reported that reduced Hb S is much less soluble than reduced Hb A Oxygenated Hb A and Hb S have the same solubility, but upon deoxygenation the solubility of Hb A falls by one half whereas that of the sickle cell hemoglobin becomes fifty times less [11] Sickling may therefore be understood as the outcome of intracellular tactoid formation in reduced sickle cells The greater the concentration of the reduced Hb S the more it is likely to form tactoids, and accordingly the tendency to sickle is more pronounced in sickle cell anemia than in sickle trait The gelling of hemoglobin inside the cells increases blood viscosity Indeed virtually all the features of the disease can be related directly or indirectly to the increase in blood viscosity which accompanies deoxygenation [32 33] The slower the blood flow the greater will be the deoxygenation and the more intense will be the transformation of the discoid red cell into the spiked sickled cell These abnormally shaped cells will be phagocytized by reticuloendothelial cells this may explain why sickle cells have a shorter life span Should the viscous bizarre shaped cells cause blockage of small vessels a vicious circle is set up Deoxygenation becomes more intense and the cell shape becomes more and more abnormal

The time factor is important in the development of sickling [34] With a normal circulation time sickle cells remain in the deoxygenated state less than the 15 sec required for sickling to begin but if for any reason they are held up in organs with a low oxygen tension there will be time for sickling A few sickled cells are invariably found in the circulating blood in the anemia

The clogging of small blood vessels is responsible for the "crises" of sickle cell anemia Infarctions which can occur in any part of the body may appear as acute abdominal emergencies pneumonia priapism heart disease or hematuria Necrotic areas in the long bones arising from the closure of nutrient vessels tend to become infected and osteomyelitis is a frequent complication A *Salmonella* osteomyelitis is almost pathognomonic of sickle cell disease The curious x ray appearance of the skull in Hb S disease is not fully explained (Fig 34 5)

On the whole and for practical purposes one can assume that the sickle cell trait is harmless However splenic infarction can occur when sickle cell trait carriers fly at high altitude in unpressurized aircraft Severe otherwise unexplained hematuria has been seen not infrequently in patients whose only abnormality was found to be a sickle cell trait [35 36]

The recognition of Hb S the studies of its biochemical and physical properties and the examination of individual patients and their families went far to support the genetic theory that sickle cell anemia is a disease of the homozygote Contradictions to this hypothesis led to the discovery

phoretic patterns of hemoglobin samples for several combinations of these hemoglobins appear in Fig 34 6

Although Hb C trait is completely harmless the homozygous state for Hb C is definitely a disease Under favorable conditions the hemoglobinopathy is latent but the decreased life span of the erythrocytes will acerbate any additional stress It is of clinical importance that sickle cell-Hb C disease and Hb C disease are associated with splenomegaly whereas in adults with sickle cell anemia the spleen cannot be felt In sickle cell anemia numerous splenic infarcts eventually lead to autosplenectomy The cells of Hb C disease are thin and numerous target cells can be seen (Fig 34 7)

Since the characterization of Hb A S and C numerous other human hemoglobins have been described Their classification is complex and will be discussed in detail further on under Nomenclature of Human Hemoglobins It may be helpful at this stage to survey what is known of the molecular changes which underlie the variations in the globin molecule

## THE POLYPEPTIDE CHAINS OF THE DIFFERENT HUMAN HEMOGLOBINS

Ingram s finding that the difference between hemoglobins A and S is in the amino acid sequence of the polypeptide chains forming the globin molecule resulted in an entirely new insight into the nature of protein polymorphism The abnormality in sickle cell hemoglobin might well have been physical and could have been caused by a difference in folding of the polypeptide chains

Indications of a difference in the free carboxylic residues had come from an ingenious differential titration by means of paper electrophoresis [41 42] It had been calculated from the difference in isoelectric point that Hb S differs from Hb A by possessing two to four more net positive charges per molecule [17] Scheinberg [41 42] brought forward evidence that the difference is due to a smaller free carboxylic group content in Hb A On electrophoresis at pH 11 7 the two pigments differ in their mobility and since lysine and arginine would lose their electrical charge at pH 11 7 the difference cannot be due to one of free amino end groups On the other hand at pH 4 and below free carboxylic groups would lose their charge and at this pH Hb A and S fail to differ on electrophoresis From this it could be concluded that Hb S carries a smaller number of aspartic or glutamic acid residues or both Such a difference of over all charge could have been due to an arrangement by which both globins were chemically identical but differed in the arrangements of the polypeptide chains so that fewer free carboxylic groups could influence the over all charge in hemoglobin S or it could have been due to a difference in chemical nature Hb S possessing a lesser number of free carboxylic

sequence is that heterozygotes for these two abnormal hemoglobin genes suffer from sickle-cell disease The anemia is modified because the total amount of Hb S in the cells is still less than that formed in the homozygous sickling condition Thus the genetic approach had led to the discovery of a

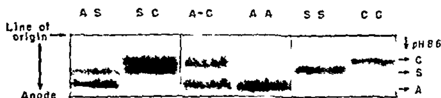



Fig 34-6 The identification of different hemoglobins by filter paper electrophoresis In an alkaline buffer hemoglobin will travel towards the positive pole The speed of migration differs adult hemoglobin (A) is faster than sickle hemoglobin (S) and S is faster than Hb C Hb D travels at the same speed as S but can be differentiated by its inability to form a gel in the reduced state (*By permission of C M Edington et al [40]*)

Fig 34-7 The red cells of hemoglobin C disease as they appear in the peripheral blood

new kind of hemoglobin under genetic control Hemoglobin C was shown to produce a symptomless trait in heterozygotes and its proportion in the trait carrying parents was less than that in the children with Hb S and C The homozygous state for hemoglobin C was not encountered by Neel, Kaplan, and Zuelzer [37] but they predicted it and it was found shortly afterwards by several other workers Ranney and her colleagues [39] were able to show that the genes for Hb A S and C were allelic Electro-

acids Seven of these were identical in both peptides but one the sixth was glutamic acid in Hb A and valine in Hb S This observation confirmed independently and indeed brilliantly the earlier predictions of Pauling [17] and Scheinberg [41 42] that the charge difference between the two proteins could be due to a loss of two carboxylic residues in Hb S, and showed that this variation was of a chemical rather than positional character The low solubility of Hb S in its reduced state was not explained by Ingram but he pointed out that the solubility of a protein depends upon the distribution of positive and negative charges upon its surface A change in surface charge pattern through the removal of a glutamic acid could well result in a change of solubility Ingram s clarification of the chemical difference between the two hemoglobins also indicated for the first time just how a gene might act in the synthesis of polymorphic proteins for the only alteration caused by the Hb S mutation was the substitution of one in 280 amino acids

When Hb C was examined by the same methods [50] it was seen that peptide No 4 was also affected The Hb A No 4 peptide had disappeared and two others had taken its place This indicated that Hb C No 4 peptide possesses an arginine or lysine in peptide No 4 where Hb A and S have no bond that can be attacked by trypsin It was then found that the glutamic acid of Hb A and the valine of Hb S are replaced by lysine in Hb C Thus a negative charge lost in Hb S is replaced by a positive ✓ charge in Hb C This difference explains the electrophoretic behavior of Hb C which on electrophoresis at alkaline pH (8 6) migrates behind Hb A toward the positive pole about twice as slowly as Hb S A change from glutamic acid to lysine involves a difference of two units of electrical charge Furthermore these findings reinforce the genetic evidence that the two mutations occur in the same place on the gene since they affect the same amino acid These findings are summarized in Table 34 1

TABLE 34-1 AMINO ACID SEQUENCE IN PEPTIDE NO 4

| Hemoglobin | β chain | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | Val | His | Leu | Thr | Pro | $Glu^-$ | Glu | Lys |
| S | Val | His | Leu | Thr | Pro | Val | Glu | Lys |
| C | Val | His | Leu | Thr | Pro | $Lys^+$ | Glu | Lys |
| G | Val | His | Leu | Thr | Pro | $Glu^-$ | Gly | Lys |

Note Val = valine His = histidine Leu = leucine Thr = threonine Pro = proline Glu = glutamic acid Lys = lysine Gly = glycine

An investigation of three samples of hemoglobin D demonstrated the fallacy of identifying a hemoglobin on the basis of its electrophoretic properties alone This potential source of error had been recognized before and indeed Hb D had been identified as one with the electrophoretic properties of Hb S but without a tendency to cause sickling in the reduced state [51] Two samples of Hb D which were otherwise indistinguishable one from a Cypriot and the other from an Indian

groups Since one molecule consists of approximately 560 amino acids, such a small difference in amino acid composition might well have been thought impossible to establish

According to the latest estimates human hemoglobin has a molecular weight of 66,700 X ray studies of horse hemoglobin have shown that the molecule is composed of two equal halves which together form an ellipsoid of the dimensions 55 × 55 × 70 A [43 44] In human hemoglobin each molecule contains four polypeptide chains of two types α and β [45, 46] The terminal sequence of the α chains is valyl leucyl, and that of the β chains valyl histidyl leucyl Since the hemoglobin molecule

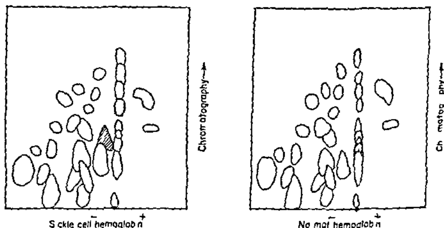



Fig 34 8 A fingerprint of the polypeptides from Hb A (*right*) and from Hb S (*left*) Electrophoresis was performed from left to right and chromatography vertically Note that the peptides all correspond except for one indicated by the shaded area (*By permission of V M Ingram* ([47]))

consists of two identical halves it would not have been necessary to look for a difference in all the 560 amino acids but only in 280 of them, but that is still a formidable number Ingram therefore decided to break down the molecule by tryptic digestion and to examine the smaller components separately [47 48] Trypsin hydrolysis breaks peptide chains where the amino acids lysine and arginine occur but nowhere else Since there are approximately 26 molecules of lysine and arginine in each half molecule of globin the digested protein resolves into 28 short amino acid chains (Fig 34-8) These peptides were separated in one direction by electrophoresis and in another by chromatography on the same large sheet of paper [49] These maps or fingerprints of the hemoglobin molecule split into small peptide components permitted detection of small differences between specific peptide fragments of Hb A and Hb S Of the 28 peptide spots all but one were identical in the two fingerprints Peptide No 4 occupied a different position in the fingerprint of the Hb S than of Hb A Ingram found that this peptide contained eight amino

Hb D and in Hb E and Hb $D_\beta$ respectively all four hemoglobins showed individual substitutions characteristic for themselves Yet a third hemoglobin D ($D_\gamma$) differed from both D and $D_\beta$ [52] (Fig 34 9)

Ingram now went further and determined in which of the two polypeptide chains $\alpha$ or $\beta$ the amino acid sequence was altered He separated

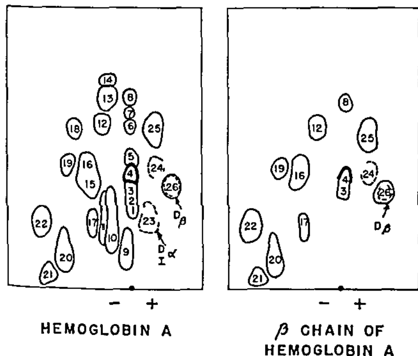
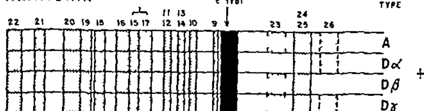
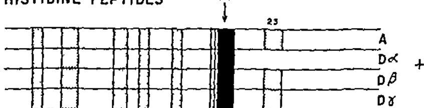


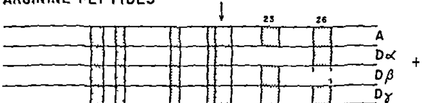

Fig 34-10 Separation of the $\alpha$ and $\beta$ polypeptide chains of normal adult hemoglobin (Hb A) On the left is the fingerprint of the globin of Hb A which is composed of $\alpha$ and $\beta$ polypeptide chains and on the right the fingerprint of the isolated $\beta$-polypeptide chain Peptides No 4 and 26 which carry the mutational characteristics of Hb S and C and of E and $D_\beta$ respectively are part of the $\beta$ chain Peptide No 23 which is altered in Hb D and I is by inference in the $\alpha$ chain (*After a sketch by Dr V M Ingram*)

the two chains by electrophoresis and submitted them each to fingerprinting (Fig 34 10) His results together with subsequent work with his collaborators [53-55] made it clear that peptide No 4 with the alterations for S and C and peptide No 26 with those for Hb I and $D_\beta$ are parts of the $\beta$ chain and that peptide No 23 which carries the differences found in Hb I and D belongs to the $\alpha$ chain Thus the $\alpha$ chains of hemoglobins A S C and I are all identical and normal and the $\beta$ chains of hemoglobins A I and D are also identical and normal

This work found full confirmation from some ingenious hybridiza



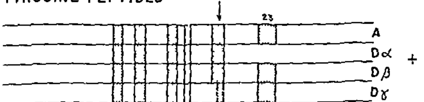




Fig 34 9 The peptides of three samples of Hb D $\alpha$ $\beta$ and $\gamma$ All three samples were indistinguishable on electrophoresis and chromatography The fingerprints however were different in all three The illustration shows schematically the results of staining the fingerprints with ninhydrin and specifically for histidine arginine and tyrosine residues respectively (*By permission of H A Itano [ 1]*)

showed a chemical difference in certain peptides Some time later when hemoglobins L and I were examined it was found that the change in the first hemoglobin D ($D_\alpha$) was found in the same peptide which was altered in hemoglobin I In the second ($D_\beta$) there was a difference from Hb A in amino acid composition of the same peptide which is altered in hemoglobin E Although the same peptide was changed in Hb I and

possess four genes a normal $\beta$ chain gene and abnormal $\alpha$-chain gene responsible for the Hb P and an abnormal $\beta$ chain gene and a normal $\alpha$ chain gene responsible for Hb S He could then form his three adult hemoglobins A S and P as follows

| | | |
|---|---|---|
| Normal $\alpha$ chain gene + | normal $\beta$-chain gene | Hb A |
| Abnormal $\alpha$ chain gene + | normal $\beta$ chain gene | Hb P |
| Normal $\alpha$ chain gene + | abnormal $\beta$-chain gene | Hb S |

There was in this case no combination of the two abnormal $\alpha$ and $\beta$ chains Indeed experiments designed to produce such a hybrid in vitro

I
II
PROPOSITUS
III

Hemoglobin A  Hemoglobin P  X Significant amounts of Hemoglobin F found

Hemoglobin S  Died very young  – No significant amounts of Hemoglobin F found

Fig 34 11 The appearance of hemoglobins A S I and F in one family from the Belgian Congo The son of a Hb A + I father and an A + S mother had Hb A S and P He married a woman with no abnormal hemoglobins and had by her both Hb A + I and Hb A + S offspring This inheritance pattern supports the theory of two adult hemoglobin genes for man One determines the $\alpha$ chain and the other the $\beta$ chain (*By permission of E W Smith et al* [*61*])

were not successful On the other hand hybrids could be formed between the non-$\alpha$ chain of Hb I and the $\alpha$ chain of Hb I using the hemoglobin of another member of this family [*63*] (Fig 34 12)

With one exception Ingram s theory has stood the test of what was known before and what has been discovered since In a case of Hb G which in this study came from an American family with Italian origins [*65*] and was probably not identical with the West African Hb G of Edington and Lehmann [*66*] the $\beta$ chain was found to be abnormal [*67*] If this is true it could not be understood on Ingram s theory that the genes for Hb S and his Hb C are nonallelic as suggested by Neel [*60*] A close

tion" experiments carried out respectively by Itano and Schroeder and their collaborators Singer and Itano [56] had observed that human hemoglobin dissociates at pH 4 3 into two unequal halves One might have expected to find the two symmetric half molecules which had been recognized in x ray studies each consisting of one $\alpha$ and one $\beta$ chain $\alpha\beta$ and $\alpha\beta$ However, at acid pH the two halves which were obtained were asymmetric and consisted of the two $\alpha$ chains and the two $\beta$ chains respectively $\alpha$ and $\beta_2$ When mixtures of Hb A and Hb S [56, 57] were submitted to this dissociation of the chains and when these were then recombined by changing the pH from acid to alkaline, the subunits of the globin molecule combined indiscriminately The $\alpha$ chains from Hb A ($\alpha_2^A$) combined both with the $\beta$ chains from Hb A ($\beta_2^A$) and from Hb S ($\beta_2^S$) and vice versa Of the four possible combinations only those with the $\beta$ chains of Hb S had the properties of sickle-cell hemoglobin regard less of whether the $\alpha$ chains were derived from Hb A or Hb S Thus both from Ingram s direct analysis and from the hybridization studies, it may be concluded that the $\alpha$ chains of Hb A and Hb S are interchangeable A logical next step was to attempt hybridization of an abnormal $\alpha$ chain with an abnormal $\beta$ chain in order to see whether this would result in a new and hitherto unobserved hemoglobin Itano succeeded in preparing such pigments by dissociating and recombining mixtures of hemoglobins I S and C [58] and was even able to combine the asymmetric subunits of human and canine hemoglobin molecules producing thereby two new hemoglobins [59]

Ingram [53] proposed a new genetic interpretation of the inheritance of human hemoglobin on the basis of these findings The possibility that there might be two hemoglobin genes had been put forward by Neel on the basis of a family study in which it was concluded that the genes for Hb S and G cannot be alleles [60] Smith and Torbert [61] found in a single individual Hb A and S, and a fast' hemoglobin Similarly Cabannes and Portier [62] found a family where Hb D and K were in herited independently and together with Hb A Dherte Lehmann and Vandepitte [63] observed a man who possessed hemoglobins S P and A and who on marrying a normal wife had children with the traits for both Hb S and Hb P Another individual with Hb A S and P was examined independently, but no family study could be carried out [64] (Fig 34 11) Ingram suggested that the two hemoglobin genes might correspond to the two peptide chains of human globin one determining the $\alpha$ chain and the other the $\beta$ chain The alterations in hemoglobins S, C, $D_\beta$ and E would then be the outcome of mutations in the $\beta$ chain gene and those seen in D I and pre um ibly P would be caused by mutations in the $\alpha$ chain gene One could then readily understand why the son of a mar riage of a sickle cell trait carrier with a Hb P trait carrier should posse s three hemoglobins (cf Fig 34 11) If he inherited Hb P and S he would

fundamental difference between adult hemoglobins and Hb F Schroeder and Matsuda [70] found that in addition to terminal valyl residues Hb F contains chains with glycyl residues ($\gamma$ chain) The valyl chain is in fact the $\alpha$ chain of normal adult hemoglobin (valyl leucyl) [71] It can now be understood why there are such profound differences between the adult hemoglobins and Hb F These differences extend to a shift in the position of the tryptophan fine spectrum band and to altered immunologic properties Unlike Hb A and S for example Hb A and F do not differ by two amino acids per molecule but rather one-half of each is composed of an entirely different polypeptide complex Certain differences in products of tryptic digestion of Hb A and F have also been observed by Katz and Chernoff [72] They observed 19 of 30 possible peptides and found that 11 of these were the same in Hb A and Hb F An abnormal fetal hemoglobin first seen at St Bartholomew s Hospital in London and called hemoglobin Bart s [73] was found to be the exact fetal equivalent of Hb H [74] It consisted entirely of the glycyl chains of hemoglobin F in the form of a tetramer Hemoglobin H ($\beta_4$) may be found without Hb Bart s ($\gamma_4$) and vice versa but a family has been studied in which the mother and one of her four children had Hb A H Bart s and F [75] More Hb Bart s was present than Hb H and whereas Hb A amounted to more than 80 per cent of the total Hb F was found only in traces This suggests that if there is a shortage of $\alpha$ chains less would be attached to $\gamma$ chains than to $\beta$ chains

On the basis of what is known of the polypeptide chains and their amino acid sequences one can now classify hemoglobins into those which possess an $\alpha$ chain and those which do not Those which do not are Hb H and Bart s Alpha chain hemoglobins can then be subdivided into those with and without a $\beta$ chain Those with a $\beta$ chain are all the hitherto examined adult hemoglobins (except Hb H) and that without a $\beta$ chain is Hb F ($\alpha_2\gamma_2$) The $\alpha_2\beta_2$ hemoglobins can be subdivided into normal adult hemoglobin and two other groups the first carrying a mutational alteration in the $\alpha$ chain (D I P Q) and the second with mutational alterations in the $\beta$ chain (S C $D_\beta$ E G J L and N) Responsible genes would be the following $\alpha$ chain gene $\beta$ chain gene $\gamma$ chain gene and at least one gene concerned with the amount of $\alpha$ chains to be produced This gene or another would also control the allocation of the $\alpha$ chains to either $\beta$ or $\gamma$ chains

## NOMENCLATURE OF HUMAN HEMOGLOBINS

It is clear that the time will come when hemoglobins will be classified and named according to their polypeptide chains and the amino acid sequences they contain Meanwhile a more empirical nomenclature will have to be used The position is not unlike that of the blood groups

examination of the experimental evidence for this nonallelism [65] suggests that this problem requires a careful study

On the basis of Ingram s theory it should be comparatively easy to determine whether an abnormal hemoglobin is due to an $\alpha$ chain or a $\beta$ chain mutation A fingerprint would show whether the alteration occurs in a peptide belonging to one of the two chains Further, hybridization with Hb S or C ($\beta$ chain mutation) would result in a new abnormal hybrid if the hemoglobin under test is the outcome of an $\alpha$ chain mutation and hybridization with Hb $D_\alpha$ or I ($\alpha$-chain mutation) similarly

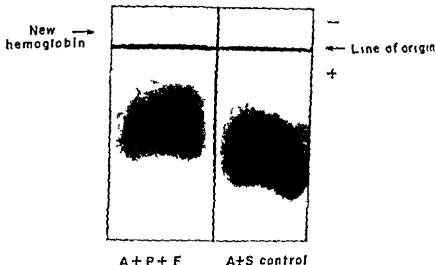



Fig 34-1. A hemoglobin hybrid The propositus of the family illustrated in Fig 34 11 possesses Hb A ($\alpha_2\beta$) Hb I ($\alpha$ $^{P}\beta_2$) and Hb F ($\alpha_2\gamma_2$) On dissociation of the peptide chains at pH 4 6 and recombination at pH 8 6 a new hemoglobin can be seen on electrophoresis presumably $\alpha$ $^{P}\gamma_2$ On the right is a hemoglobin A + S control (*By permission of E W Smith et al [61]*)

would give a new abnormal hybrid if the test hemoglobin is the result of a $\beta$-chain mutation Such experiments have by now been carried out with Hb J N I O K Norfolk and Q and have indicated that the first four must carry their abnormality of amino acid sequence in the $\beta$ chain the others belong to the hemoglobins with abnormal $\alpha$ chains [155]

Yet another type of abnormality has been established in the case of Hb H It differs from the other adult hemoglobins in that it is absent from the blood of parents of persons with Hb H, but it is occasionally found in a sibling or grandparent It does not seem to appear unless a gene for thalassemia is also present [69] Jones et al [69] have recently reported that Hb H consists entirely of normal $\beta$ chains Since its molecular weight does not differ from that of Hb A the $\beta$ chains must be associated in a tetramer

Investigation of the polypeptide chains has also thrown light on the

All the hemoglobins (with the possible exception of hemoglobin M) have been discovered because they showed electrophoretic properties different from hemoglobin A It must be expected that there are many more in which substitutions in the peptide chains do not alter their electric charge and also that there may be hemoglobins with identical electrophoretic properties which do not have the identical amino acid sequence Indeed this has already been shown for Hb D for when other wise identical samples were submitted to an analysis of their peptides after tryptic digestion [52] sequence differences were observed Electro phoretic differences are often small and it has been suggested that some

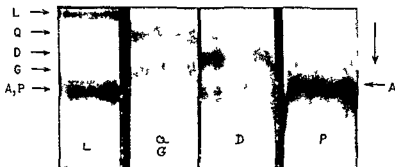



Fig 34-13 Separation by resin chromatography (Amberlite IRC 50 pH 6) of hemo globins which on electrophoresis at pH 8 6 move similarly or identically In this instance chromatography was used to identify an unknown hemoglobin C3656 as Hb G The order of electrophoretic mobility at pH 8 6 A Q G L = P D is on chroma tography A = P G D Q L Hb P and Hb A form a broad band (*By permission of J A Hunt [14 ]*)

of the electrophoretically abnormal variants might be artifacts [93] but when the variants occur in families the evidence is convincing for a new type of hemoglobin Electrophoresis at varying pH and chromatography on Amberlite IRC 50 [94] have been useful in differentiating hemo globins which have closely similar electrophoretic properties at alkaline pH A chromatographic comparison of several hemoglobins appears in Fig 34 13

Ager Lehmann and Vella [89] have proposed the following classifica tion as a temporary solution They divided the human hemoglobins into two major groups fetal and adult The fetal group which shows an ultraviolet spectrum of the fetal type includes Hb F the pigment of Fessas and Papaspyrou [95] $\gamma_4$ ( Bart s ) and Alexandra The adult variants are divided into six groups according to their behavior on paper electrophoresis at alkaline pH These groups can be established by using as markers normal hemoglobin and Hb $\beta_4$ (H) and S Erythrocytes con taining Hb $\beta_4$ can be identified independently of electrophoretic methods because cells containing this hemoglobin form inclusion bodies upon

Although much information has been gained about the chemical nature of the agglutinogens A and B, the H substance and the Lewis substance which coats the red cells blood groups are still identified according to their agglutinins

In 1953 Chernoff and his colleagues [76] proposed that for mnemonic reasons the following letters be assigned to hemoglobins known at the time

- F Fetal hemoglobin
- A Normal adult hemoglobin
- S Sickle cell hemoglobin

Later Singer [77] proposed that the letter M should be used for the abnormal methemoglobin of Horlein and Weber [15] All other hemoglobins which might be discovered later were to be known by letters of the alphabet in the order of their discovery beginning with the letter C (b or B had at one time been used for the sickle cell hemoglobin) Difficulties arose when different hemoglobins were given the same letter by their discoverers or when two or more designations were suggested for the same hemoglobin Thus hemoglobin $A_2'$ is still being called $A_4$ by some investigators [14] and $B_2$ by others [78] It is doubtful whether Hb G discovered in West Africa [66] is the same as Hb G discovered by Schwartz and his colleagues [65] Cabannes and Buhr [79] named a 'fast' hemoglobin discovered by them in Algeria Hb I not knowing that this letter had been allotted by Rucknagel and his colleagues [80] to a hemoglobin which they had originally named Hb H [81] Rucknagel and coworkers had not been aware that the letter H had been given to a newly discovered hemoglobin still known by this letter today [82] Cabannes et al [83] altered their designation from I to J, but then found that this letter had been given to another newly discovered hemoglobin [84] Eventually it was decided at the Sixth Congress of the International Society for Hematology to rename this hemoglobin for a third time and it has since been known as Hb K [85] In 1956 Robinson et al [86] suggested a way out of this dilemma by naming two hemoglobins 'Liberian I' and 'Liberian II' after the place where they had been discovered Later these were designated Hb N and K [78] In this way Hb P was first known as the 'Galveston type [87] and Hb O as 'Buginese X' [88] Hopkins I' and 'Hopkins II seen at Johns Hopkins Hospital in Baltimore [61] are presumably hemoglobins I and J Hemoglobin Norfolk [89] hemoglobin Sud Vietnam [90] and Stanleyville I and 'Stanleyville II' [91] are other examples Two abnormal fetal hemoglobins have been named after the hospitals where they were first seen Bart s after St Bartholomew s Hospital in London [73] and Alexandra after the hospital of that name in Athens [92] For Bart s the designation of $\gamma_4$ will have to be used in the future [74]

and well known hemoglobins by their present names S C D F etc but the more obscure pigments might well be labeled by giving them a group number or letter followed by a small letter If after such a scheme was adopted a hemoglobin was found which moved for example between hemoglobins E $A_2$ on the one hand and hemoglobin O on the other (C group 3a and 3b and C group 4) it would be classified as C group $^3/_4$ If one wished to place a hemoglobin between the hypothetical pigment C group $^3/_4$' and O ( C group 4 ) it could be designated C group $^3/_4$//4

## VARIANTS OF FETAL HEMOGLOBIN

### PREFETAL HEMOGLOBIN

It has been claimed that just as Hb F precedes Hb A in human development so another more primitive hemoglobin precedes Hb F in the fetus There is no evidence that Hb F precede the adult pigment in the true sense of the word for although the proportion of Hb F is higher in the embryo than at birth Hb A has been found in the youngest embryos examined

There is evidence both in favor of and against the existence of a prefetal primitive hemoglobin Allison s primitive hemoglobin P [97] was not a human hemoglobin but was found in rat embryos [98] In man investigation is difficult because of the small amount of blood which can be obtained from embryos when they become available Halbrecht and Klibanski [99 100] found in embryos an alkali resistant hemoglobin with a lower mobility on paper electrophoresis at pH 8 6 than that of either Hb F or A Kunzer and Drescher [101 103] also found a primitive alkali resistant hemoglobin with an ultraviolet spectrum of the Hb F type Other workers have failed to find either in embryonic or cord blood any hemoglobin other than A and F [104–106]

### HETEROGENEITY OF FETAL HEMOGLOBIN

Vella [107] found in addition to a principal fraction two small components of hemoglobin in cord blood Since his observations were made by paper electrophoresis it cannot be said at present in which way they are related to minor fractions observed on chromatography [94 108–110] Indeed chromatographic results are not entirely consistent among themselves Vella considers the two small fractions the same as those seen by him and others on occasion in greater quantity and described as abnormal fetal hemoglobin One is the fast moving pigment of Fessas and Papaspyrou [95] and the other hemoglobin Alexandra [92] Thus Hb F would appear to be heterogeneous just as is Hb A where in addition to the main component there are smaller fractions $A_2$ and $A_3$ Traces of Hb Alexandra can be found in all cord bloods but an increased

incubation with cresyl blue The electrophoretic properties are summarized in Fig 31 14 Sickle cells can also readily be identified upon incubation under reducing conditions Hemoglobin A is identified as that occurring in a number of normal controls With the help of Hb A $\beta_4$ (H) and S six groups of adult hemoglobins can be established These

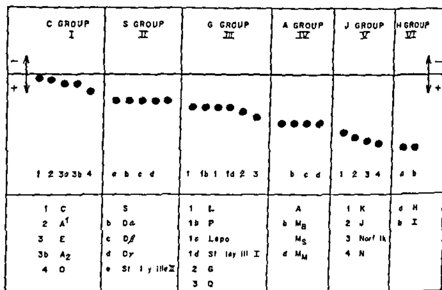



Fig 31 14 A diagram showing the pattern of adult hemoglobins on paper electrophoresis at pH 8 6 (*By permission of A L Ogden et al and the Honorary Editors of the Proceedings of the Royal Society of Medicine [96]*)

may be numbered I II III etc or they may be given the name of the first hemoglobin discovered within the group

| | | |
|---|---|---|
| Group I | Hemoglobins moving more slowly than S | C group |
| Group II | Hemoglobins moving like S | S group |
| Group III | Hemoglobins moving between S and A | G group |
| Group IV | Hemoglobins moving like A | A group |
| Group V | Hemoglobins moving between A and H | J group |
| Group VI | Hemoglobins moving like H | H group |

Within these six groups hemoglobins could again be numbered according to their mobility or when they have the same mobility they could be given (according to the time of their discovery) the additional letters a b c etc

When such a scheme is accepted a newly discovered hemoglobin could then be allotted to one of the six groups Clearly a digit should be assigned only after full comparison and international agreement It is obvious that it would remain the general practice to refer to the common

3 *Hemoglobin Bart's* ($\gamma_4$) On electrophoresis at pH 8 6 this hemoglobin [73 74] moves faster than J and more slowly than H similarly to Hb N On chromatography at pH 6 it moves fastest of all known hemoglobins It was thought to be an abnormal fetal hemoglobin because of its absence in both parents because of its resistance to alkali denaturation (intermediate between those of Hb A and Hb F) and because of its ultraviolet spectrum which was of the Hb F type Hemoglobin Bart's disappears as the child grows older It has been seen in approximately 50 cord bloods in various parts of the world

There are now also records of Hb Bart's in an older child and in adults The first was a Greek boy with thalassemia [112] The two adults were

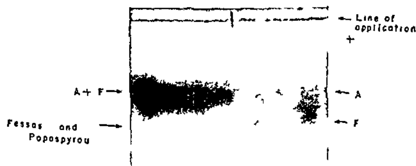



Fig 34 15 The Fessas and Papaspyrou abnormal hemoglobin Paper electrophoresis at pH 8 6 On the left cord blood and on the right Hb A and J The fast moving hemoglobin in the cord blood moves similarly to Hb J Its ultraviolet spectrum is of the Hb F type

a Jewess from Baghdad and her daughter [7?] In these patients Hb Bart's ($\gamma_4$) was found together with Hb H ($\beta_4$) so that these two women exhibited a dual $\alpha$ chain deficiency in adult and fetal hemoglobin One might expect such a dual deficiency to occur more often and there are indications that this might indeed be the case Huisman [115] found that Hb H resembles Hb F in amino acid composition particularly in its high isoleucine content (A 0 03 per cent F 1 85 per cent and H 1 2 per cent) Since the $\alpha$ chains of Hb F and Hb A are identical one may presume that the higher isoleucine content of Hb F is due to its $\gamma$ chain Since Hb H is now known to be the tetramer of the $\beta$ chain [69] Huisman's observation is difficult to understand His isolation of Hb H was by electrophoresis on a starch slab a method in which Hb Bart's at the time not yet described might well fuse into Hb H If Huisman's preparation of Hb H also contained an undetected admixture of Hb Bart's he would have found a fetal type of amino acid composition in his analysis

A report from Thailand [116] mentions two fast hemoglobin components found in thalassemia hemoglobin H disease and states that the second component resembles Hb Bart's but the details have not yet appeared

amount would be considered an abnormality just as an increased Hb $A_2$ is considered abnormal

## ABNORMAL FETAL HEMOGLOBINS

Fessas and Papaspyrou [95] observed in an infant a fast moving hemoglobin which disappeared as the child grew older This pigment was described as moving at alkaline pH on paper electrophoresis between A and J At the same time Vella observed a hemoglobin in cord blood which was sent to Athens and considered by Fessas to be identical with the pigment he had described This hemoglobin was then referred to as the hemoglobin of Fessas and Papaspyrou in order to differentiate it from another faster moving fetal hemoglobin Hb Bart's Recently Fessas has observed more examples of a fast hemoglobin in cord blood and although a comparison with his original specimen is no longer possible he now considers that this hemoglobin was in fact identical with Hb Bart's and not with the hemoglobin seen in Singapore by Vella [107] The designation of a hemoglobin which unlike Hb Bart's moves more slowly than Hb J on electrophoresis at pH 8 6 as "hemoglobin of Fessas and Papaspyrou" may therefore have to be abandoned The question of renaming Hb Bart s does not need to arise since this has now been identified as the $\gamma_4$ hemoglobin (i e a tetramer of $\gamma$ chains) and it will probably be referred to as such in the future [74]

Three hemoglobins other than F are thus known which exhibit features similar to those of Hb F The first two may possibly be present in normal cord blood but only in low quantity

1 *Hemoglobin of Fessas and Papaspyrou* [95 107] This is the least well-defined abnormal fetal hemoglobin On paper electrophoresis at pH 8 6 it moves faster than Hb A but more slowly than Hb J and on chromatography at pH 6 it moves between Hb F and H The ultraviolet spectrum is of Hb F type Investigators either have not found it to be alkali resistant [95] or have not examined it for alkali resistance [107 112] Recent observations have been made in Indonesia [113] and in Nigeria [114]

2 *Hemoglobin Alexandra* [92] This hemoglobin moves on paper electrophoresis at alkaline pH more slowly than Hb S and faster than Hb C i e it is similar to the Hb O (Fig 34 15 34 16) Unlike Hb O it behaves chromatographically on agar electrophoresis at pH 6 like Hb F, and moves faster than Hb A The ultraviolet spectrum is of the Hb F type It is more resistant to alkali denaturation than Hb A

This hemoglobin has been described twice once in a Greek and once in a Chinese infant If there should be a connection between the observations of a slow moving prefetal hemoglobin [99] and the so called abnormal fetal hemoglobins it is most likely that this would relate to Hb Alexandra

Gamma$_4$ hemoglobin was originally discovered in an infant of mixed Caucasian Negro Chine e extraction The second child observed in Britain was also of West Indian origin and the parents showed both Negroid and Mongoloid features [117] Further instances were seen in a sample of cord blood from Nigeria [114] and in one from a Negro infant from Texas [118] Two cases of $\gamma_4$ were observed in Chinese infants in Singapore [107] In Thailand 22 of 415 cord bloods contained this abnormal fetal component (two were twins) [116] It was in this study that

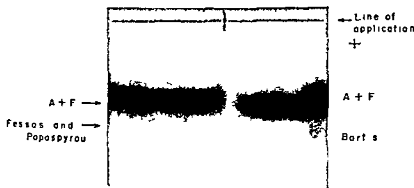



Fig 31-17 Two fast moving abnormal fetal hemoglobins On the left is the hemoglobin of Fessas and Papaspyrou and on the right hemoglobin Bart s Paper electrophoresis at pH 8 6

evidence for a possible association between the two $\alpha$ chain deficiencies was presented but the authors also record that they have seen one infant with only one $\gamma_4$ whereas a sister showed only the other $\beta_4$

Most of the cases of $\beta_4$ have been recorded in Chinese and in Greeks It is remarkable that the same association occurs both for Hb Alexandra and for $\gamma_4$ The only Caucasians with $\gamma_4$ so far seen have been Greeks In addition to the Greek boy with thalassemia already mentioned [112] Fessas [119] states that he has seen numerous instances of this hemo globin in Athens and indeed as stated above considers that his first report [9?] of an abnormal fetal hemoglobin was one of $\gamma_4$ rather than of the other fast abnormal fetal hemoglobin which has hitherto been re ferred to as the Fessas and Papaspyrou component (Figs 31 17 31 18)

## ADULT HEMOGLOBINS

*Widely Occurring Variants* A S *C D I*

*Hemoglobin* A is the normal adult hemoglobin Its own peculiar abnormality is thalassemia which is the outcome of a reduced ability to form Hb A and does not relate to any of the other hemoglobins It is of course possible that what is now considered to be a single hemoglobin A

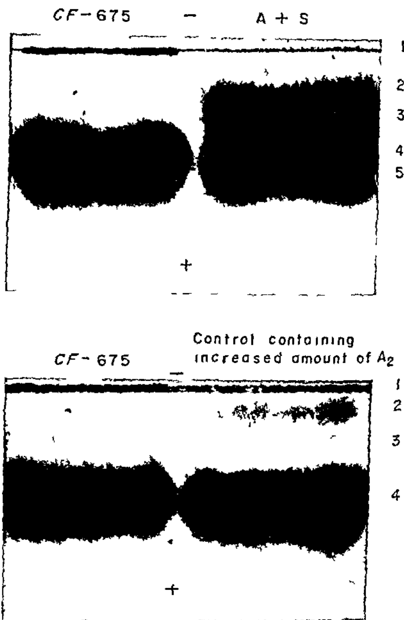



Fig 34-16 Hemoglobin Alexandra moves on electrophoresis at pH 8 6 *exactly like* hemoglobin O i e between S and E (or A) It can be differentiated from hemoglobin O by chromatography at pH 6 and on agar electrophoresis at pH 6 2 *By these* two methods it shows a greater mobility than A whereas O moves behind A In addition Alexandra has a fetal type of ultraviolet spectrum *(By permission of J A M Ager et al [89])*

mia [123] until with the introduction of electrophoretic technique some for example were found to be Hb C disease [123] Thus whether there is a deficiency of Hb A per se as in thalassemia or whether Hb A has been replaced by another hemoglobin the resulting clinical pictures are remarkably alike An exception of course is sickle-cell disease with its additional complications due to intravascular infarcts There is other evidence suggesting that a deficiency of Hb A rather than the presence of other hemoglobins causes microcytosis Whereas trait carriers are symptomless double heterozygotes for an abnormal hemoglobin and for

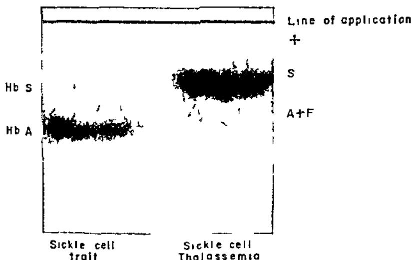



Fig 34 19 Sickle-cell trait and sickle-cell thalassemia The sample on the left shows the sickle-cell trait and that on the right the hemoglobin of a double heterozygote for Hb S and thalassemia respectively Hb A formation is suppressed when thalassemia is present

thalassemia (in whom the proportion of Hb A is lower than in simple abnormal hemoglobin trait carriers) resemble clinically homozygotes for the appropriate abnormal hemoglobin Thus in the case of sickle cell trait they present with sickle-cell thalassemia disease [124–132] and in that of hemoglobin C trait with hemoglobin C thalassemia [133 134] (Fig 34 19) The clinical condition approaches that of the homozygous state more nearly when the relative concentration of the abnormal hemoglobin is higher This rule is not universal Hemoglobin E disease tends to be milder than the combination of Hb E trait with thalassemia [135–139] This is true even though in Hb E thalassemia more commonly than in any other of the doubly heterozygous conditions the suppression of Hb A production is complete In an Indian family where Hb J and thalassemia were found [140] a double heterozygote showed no suppression of Hb A formation and the proportion of A to J was the

could be a group of different hemoglobins with single amino acid substitutions *which do not affect electrophoretic mobility* Ingram et al have in fact suggested that the hemoglobin A in thalassemia might be an abnormal hemoglobin of this type, and that there might be α chain substituted and β chain substituted hemoglobins A" [*120*] This is not impossible because it is known that otherwise indistinguishable samples of hemoglobin D possess different amino acid sequences [*59*] The abnormality in thalassemia would be in $A_1$ the main component If Ingram's theory of an abnormal hemoglobin indistinguishable at present from Hb A is confirmed then thalassemia minor would be an example of a disability due to an abnormal hemoglobin inherited together with

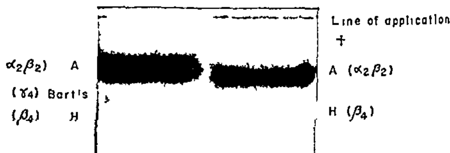



Fig 34 18 A dual α-chain deficiency Comparison by paper electrophoresis at pH 8 6 of a sample containing Hb A and H with one from a woman with Hb A F (trace) Bart s and H These hemoglobins may alternately be referred to as $\alpha_2\beta_2$ $\alpha$ $\gamma_4$ (trace) $\gamma$ and $\beta_4$ (*By permission of* I M *Katz et al* [*72*])

normal Hb A With certain qualifications for the sickle cell trait the concept holds at the present time that a trait for an abnormal hemoglobin is harmless

With one exception the homozygous state for Hb G [*121*] all homozygotes for one abnormal hemoglobin gene and all double heterozygotes for two different abnormal genes have clinical manifestations of the abnormality It seems that the absence of Hb A is associated with a flattened cell and a low mean cell hemoglobin content The abnormality results in a *decreased* osmotic fragility because the resistance of a red cell to hydration is largely a measure of the difference between the volume of the cell and that of a sphere of equal surface [*122*] There is invariably a shortened life span although this investigation has not yet been carried out in all these disorders with equal thoroughness It may be assumed that this abnormality which is common to all these conditions has a common denominator the absence or shortage of Hb A It is noteworthy that in thalassemia in which (as it may still be assumed) Hb A is the only adult hemoglobin the pathologic picture is much the same as in the hemoglobinopathies due to abnormal hemoglobins A number of homozygous conditions were in fact thought to be instances of thalasse

tion in the No 4 peptide of the fingerprint the alteration in Hb E occurs ✓ in the No 26 peptide (Fig 34 21) The amino acid change explains its electrophoretic mobility at pH 8 6 but not its behavior at pH 6 5 One might conclude that the *position* of an amino acid substitution in the molecule would influence the electrophoretic behavior as well as the

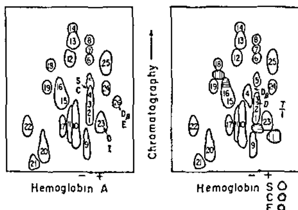



Fig 34 21 A summary of the mutational changes as se n in the pepti l finger prints of Hb S C and E (*By perm is i n of S B ze et al [ ]*)

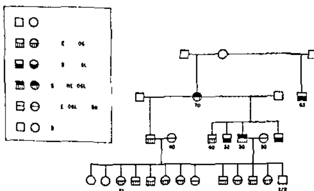


Fig 34 The inl ritance pattern in a family with Hb A S and E For further discus n see text (*By perm issi n of th B it M J 2 1958*)

change in electrical charge Hemoglobin S and C which have their amino acid substitution *in the same amino acid link of the β chain are* allelic characters [39] There is only one record of a family in which Hb S and E occurred together [146] There was conflicting evidence as regards the inheritance of the two hemoglobins (Fig 34 22) The son of a woman with hemoglobins S and E presented the same hemoglobin combination The father had died This finding would exclude that the genes for Hb S and E are alleles only *if* the father had been proved not

same as in persons with nonthalassemic Hb J trait There was also no evidence of a hemoglobinopathy ("hemoglobin J thalassemia") in this person

*Hemoglobin D* was first found in a patient with atypical hemolytic anemia Electrophoresis revealed a single hemoglobin migrating in the position of Hb S [*141 142*] One of the parents failed to show sickle cells on repeated tests but on electrophoresis a sickle-cell trait pattern was found It was then discovered that a hemoglobin with the electrophoretic

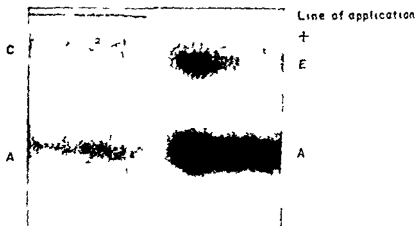



Fig 34-20 A comparison of Hb C and Hb E traits Hb E moves slightly faster than Hb C Paper electrophoresis at pH 8 6

properties of Hb S but without its abnormally low solubility in the reduced state was responsible for the conflicting observations The propositus was in fact suffering not from sickle-cell anemia but from sickle cell-hemoglobin D disease The parent was a Hb D trait carrier At one time thought to be a rare hemoglobin D is now known to occur in 2 per cent of Punjabis and in 1 per cent of Lohana speaking Gujeratis in southern India [*143*] It is now known that "hemoglobin D" is not a single compound but that in this electrophoretically and otherwise identical group several pigments with identical charge but different peptide sequence are included [*52*]

*Hemoglobin E* was discovered in a family in California [*144*] and independently in Thailand [*135 145*] The observations in Thailand provided the first evidence that abnormal hemoglobins other than Hb S exist at high frequency in non Negroids On electrophoresis at alkaline pH Hb E is very similar to Hb C but unlike C it is similar to Hb S on electrophoresis at acid pH (Fig 34 20) The mutational change in Hb E is in the $\beta$ chain and as in Hb C involves the substitution of a glutamyl residue of Hb A by a lysyl residue The peptide affected is however not the same in both cases Whereas for Hb C (and S) there is an altera

[*148*] This subject is discussed fully in Chap 33 The variants are named after the localities where they were found $M_{B(oston)}$ $M_{S(askatoon)}$ $M_{A(ugusta)}$ Inheritance follows simple Mendelian rules and the affected individuals are thought to be heterozygotes

*Hemoglobin H* is now known to be $\beta_4$ This deficiency of the alpha chain does not become apparent unless there is also a gene for thalassemia [*68*] The hemoglobin was discovered in 1955 in a Chinese family [*62*] and also

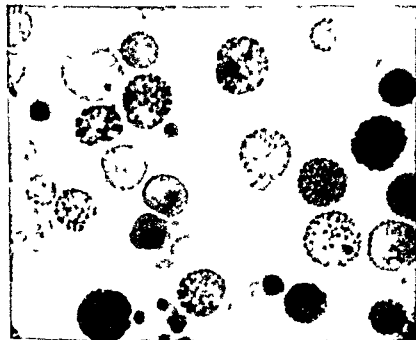


Fig 34-23 Inclusion bodies in cells containing Hb H The cells were incubated at 37 C with isotonic cresyl blue The large inclusions which give the cells almost a nucleated appearance are giant inclusions seen in patients after splenectomy

in Greece [*149*] It has little stability and denatures on storage in the cold On incubation with cresyl blue red cells containing this hemoglobin form numerous blue inclusion bodies (Fig 34 23) These probably represent small foci of denatured hemoglobin

On paper electrophoresis at pH 8 6 Hb H and I are the fastest moving pigments At pH 6 5 Hb H is the only known hemoglobin which migrates toward the anode

*The Lepore hemoglobin* (named after the family in which it was discovered) [*150*] was found during family studies of cases with thalassemia major No other examples outside this family have yet been reported Both parents were of Italian extraction The propositus an infant ap-

to carry the sickle cell trait, and about one fifth of the population to which this family belonged are sickle cell trait carriers On the other hand, in the next generation four children of the man with the two ab normal hemoglobins and of his normal wife had only one abnormal hemoglobin each *Thus on the whole the evidence favors allelism or* close linkage of the genes for Hb E and S Hunt and Ingram [147] state that the locus of the Hb E mutation is probably some distance away from that of the Hb S locus in the gene of hemoglobin Nevertheless they may be closely linked in the usually accepted genetic sense The specific chain alterations of Hb A and E are as follows

Hb A—Val Asp Val Asp Glu Val Gly Gly Glu Ala Leu Gly Arg
Hb E—Val Asp Val Asp Glu Val Gly Gly Lys Ala Leu Gly Arg

The known combinations of Hb S C D and E appear in Table 34 2

TABLE 34 2 KNOWN COMBINATIONS OF HEMOGLOBINS S C D AND E

| *Disease or trait* | *Genotypes* | *Hemoglobins found* |
|---|---|---|
| Asymptomatic traits | | |
| Sickle-cell trait | AS | A + S |
| Sickle-cell hemoglobin G trait | GS | G + S |
| Hemoglobin C trait | AC | A + C |
| Hemoglobin D trait | AD | A + D |
| Hemoglobin E trait | AE | A + E |
| *Sickle-cell hemoglobin I trait* | IS | S + A + P + (F) |
| Sickle-cell diseases | | |
| Classical sickle-cell anemia | SS | S + F |
| Sickle-cell thalassemia | AS + thal | S + A + F |
| Sickle-cell hemoglobin C disease | SC | S + C + (F) |
| Sickle-cell hemoglobin D disease | SD | S + D |
| Sickle-cell hemoblogin E disease | SE | S + E |
| Sickle-cell hemoglobin J disease | SJ | S + J |
| Sickle-cell hemoglobin N disease | SN | S + N |
| Thalassemias | | |
| Sickle-cell thalassemia | AS + thal | S + A + F |
| Hb C thalassemia | AC + thal | C + A + (F) |
| *Hb D thalassemia* | *AD + thal* | D + F |
| Hb E thalassemia | AE + thal | E + F |
| Nonsickling nonthalassemic diseases | | |
| Hemoglobin C disease | CC | C + (F) |
| Hemoglobin D disease | DD | D |
| Hemoglobin E disease | EE | E + (F) |

Note (F) = F irregularly found

*Hemoglobins Associated with a Hemoglobinopathy in the Heterozygote*

*Hemoglobin M* originally described in 1948 [15] is now known to occur in three variants all distinguished by their spectrum in the visible region

*Hemoglobin Q* was seen in a Chinese family [*158*] The first patient also had Hb H In this patient who suffered from a thalassemia like condition Hb Q replaced the A which is usually seen with Hb H The only other person observed was the mother of the propositus who showed a hemoglobin mixture of 60 per cent Hb A and 40 per cent Hb Q The mutational change in Hb Q is in the $\alpha$ chain Hb Q moves on electrophoresis at pH 8 6 just faster than Hb G and is again best differentiated from similar slow hemoglobins by chromatography at pH 6 (cf Fig 34 13)

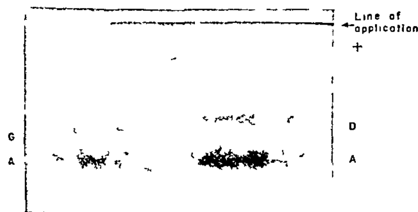



Fig 34-24 Hemoglobin G on paper electrophoresis at pH 8 6 Hb G moves faster than S and D but separates clearly from Hb A

*Stanleyville I and II hemoglobins* were seen in a survey in the Belgian Congo [*91*] Stanleyville I resembles Hb L and P on electrophoresis at pH 8 6 but moves on chromatography at pH 6 like Hb D Stanleyville II moves on electrophoresis like Hb D but moves on chromatography behind Hb D Both hemoglobins were found in the extreme northeast of the Belgian Congo the first in Nilotes and the second in a Bantu and in a partially Bantu partially Greek family living in a Nilotic area

## *Rare Fast Hemoglobins*

*Hemoglobin I* was seen for the first time in an American Negro family where it was present in three generations [*87*] It has always been seen in people of at least partial Negro de cent It is probable that a hemoglobin described as Hopkins I [*61*] is an example of this pigment This hemoglobin has its mutational alteration in the $\alpha$ chain in the same finger print peptide as Hb D peptide No 23 Hemoglobin I moves on electrophoresis at alkaline pH like Hb H but unlike Hb H moves at pH 6 5 toward the negative electrode

*Hemoglobin J* was first seen in a young American Negro woman [*84*] whose father and six siblings also carried the abnormality Further

parently suffering from thalassemia major was found to possess in addition to Hb A 74 per cent Hb F 1 2 per cent Hb $A_2$ and 5 per cent Hb Lepore Since the mother possessed 11 per cent of Hb Lepore and the father had thalassemia minor with a Hb $A_2$ concentration of 5 5 per cent, it was concluded that the child suffered from a combination of the two abnormalities Four other members of the family showed the Lepore hemoglobin in combination with Hb A and a slightly raised level of Hb F (2 3 to 2 6 per cent), they were considered to be heterozygotes

The presence of some Hb F suggests that the Lepore trait is not simply a genetic replacement of part of Hb A Gerald and Diamond [150] consider that the Lepore trait is more related to thalassemia than to the abnormal hemoglobin syndromes It may be that Motulsky s interpretation of Hb H penetrance in association with thalassemia [68] is paralleled by the Hb Lepore trait, and that this pigment is seen in the phenotype only when a gene for thalassemia is also present It has indeed been suggested that Hb $\alpha_4$ which would correspond to $\beta_4$ (H), might turn out to be the Lepore hemoglobin The only efficient separation from Hb A has been achieved on starch slab electrophoresis at pH 8 6

### *The ' Rare Slow Hemoglobins '*

*Hemoglobin G* moves between A and S but separates from A when seen in the trait (Fig 34–24) This hemoglobin was first seen in a West African family [121] it may or may not be the same as the Hb G found in a family of Mediterranean extraction [65] which does not separate from Hb A on electrophoresis at pH 8 6 This latter hemoglobin has its mutational alteration in the $\beta$ chain next to that where the S and C substitutions occur [67] A hemoglobin with the electrophoretic and chromatographic properties of Hb G has also been seen in a Chinese woman [151] Hemoglobin G is remarkable for not causing any abnormality in the homozygote

*Hemoglobin L* moves on electrophoresis at pH 8 6 slightly behind G and in front of S It was discovered in a family of North West Indian origin [152] and has since been found on seven different occasions always in Indians [153 154] Fingerprint studies have shown it to carry its mutational change in the $\beta$ chain [155]

*Hemoglobin O* which moves on electrophoresis at pH 8 6 between E and S has been seen in 16 of 775 blood samples [26] from Buginese living on the Celebes (Bulawesi) [156] Family studies have not been possible

*Hemoglobin P* was first seen in American Negroes [57 157] and has since been seen repeatedly in the Belgian Congo [63 64] It has the same electrophoretic properties at pH 8 6 as Hb I but can be differentiated from it by chromatography at pH 6 (cf Fig 34 13) Its mode of inheritance as well as other evidence, suggest that its mutational alteration is situated in the $\alpha$ chain [63]

*Hemoglobin Q* was seen in a Chinese family [*158*] The first patient also had Hb H In this patient who suffered from a thalassemia like condition Hb Q replaced the A which is usually seen with Hb H The only other person observed was the mother of the propositus who showed a hemoglobin mixture of 60 per cent Hb A and 40 per cent Hb Q The mutational change in Hb Q is in the α chain Hb Q moves on electrophoresis at pH 8 6 just faster than Hb G and is again best differentiated from similar "slow" hemoglobins by chromatography at pH 6 (cf Fig 34 13)




Fig 34-24 Hemoglobin G on paper electrophoresis at pH 8 6 Hb G moves faster than S and D but separates clearly from Hb A

*Stanleyville I and II hemoglobins* were seen in a survey in the Belgian Congo [*91*] Stanleyville I resembles Hb L and P on electrophoresis at pH 8 6 but moves on chromatography at pH 6 like Hb D Stanleyville II moves on electrophoresis like Hb D but moves on chromatography behind Hb D Both hemoglobins were found in the extreme northeast of the Belgian Congo the first in Nilotes and the second in a Bantu and in a partially Bantu partially Greek family living in a Nilotic area

*Rare Fast Hemoglobins*

*Hemoglobin I* was seen for the first time in an American Negro family where it was present in three generations [*81*] It has always been seen in people of at least partial Negro descent It is probable that a hemoglobin described as Hopkins I [*61*] is an example of this pigment This hemoglobin has its mutational alteration in the α chain in the same finger print peptide as Hb D peptide No 23 Hemoglobin I moves on electrophoresis at alkaline pH like Hb H but unlike Hb H moves at pH 6 5 toward the negative electrode

*Hemoglobin J* was first seen in a young American Negro woman [*84*] whose father and six siblings also carried the abnormality Further

examples have been seen in Indians Indonesians and West African Since identification is based solely on electrophoretic properties one might suspect that these widely distant places have given rise to different hemoglobins with the over all charge of hemoglobin J but with different peptide sequences Hb J moves faster than Hb A and Hb K on electro phoresis at pH 8 6 and does not separate from A at pH 6 5 (Fig 34 25)

*Hemoglobin K* moves on electrophoresis at pH 8 6 just in front of Hb A and barely separates from it It was first seen in Algeria [79] and has also

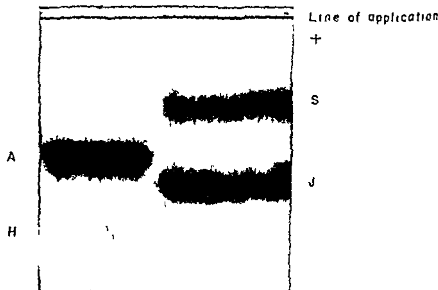



Fig 34-25 Comparison by paper electrophoresis at pH 8 6 of Hb A + H sample with one containing Hb J and S Note that the distances S-J and A H are the same (*The Hb J + S sample was kindly supplied from the first case of this condition seen by Drs J E MacIver and L N Went University of the West Indies Kingston Jamaica*)

been seen in Liberia where it was described as Liberian II [88] It has since been een in other West Africans in Southern Indians and in Bengalis It is possible that Hopkins II is Hb K This hemoglobin was inherited independently from hemoglobin S and its mutational change is in the $\alpha$ chain

*Hemoglobin N* was first seen in Liberia [86] and for a time "Liberian I" was thought to be Hb J It moves only slightly faster than Hb J on paper electrophoresis at pH 8 6 but differs from Hb J by separating from Hb A at pH 6 5 and also on chromatography at pH 6 [73] (Fig 34 26) Hb N has since been seen in a few instances in Portuguese territories in the northern parts of West Africa It is possible that a hemoglobin provisionally described as "R by Chernoff and found by him in association with Hb S is also Hb N Hb N has also been seen in association with Hb S in West Africa The mutational change is in the $\alpha$ chain

*Hemoglobin Norfolk* was observed after Hb Q and after the letter R was claimed provisionally for what might in fact have been an instance of Hb N It was therefore decided to wait with giving the new hemoglobin a letter and it has been named after the county from which the family showing this fast moving hemoglobin was derived This is an example of an abnormal hemoglobin in an English family with no known foreign connections Hb Norfolk [89] resembles Hb J on electrophoresis at pH 8 6 and moves only slightly faster though it definitely moves more

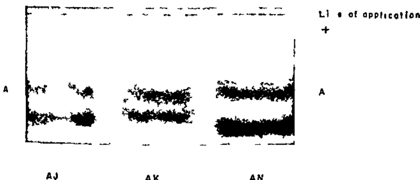



Fig 34 26 Hemoglobins J K and N on paper electrophoresis at pH 8 6 These hemoglobins move faster than Hb A in the order N J K

slowly than Hb N Hb Norfolk differs from Hb J by separating from A on electrophoresis at pH 6 5 This hemoglobin was discovered in a British airman in Singapore where it was the only abnormal European sample among 2 550 samples It was then found in the father and in his brother in Norfolk England Its mutational change is in the $\alpha$ chain √

## WORLD DISTRIBUTION OF ABNORMAL HEMOGLOBINS

Anthropologists are particularly interested in human characters which are not so rare as to be of no general importance nor so widespread that they lose their value in distinguishing among different human populations For anthropologic purposes perhaps the ideal characters among the abnormal hemoglobins are Hb C and E Both pigments are present in millions of human beings but at the same time they are peculiarly associated only with certain well-circumscribed groups of people [159] Hb C discovered in American Negroes could be expected to have come with their ancestors from West Africa but it was surprising to find that Hb C which is not frequent in American Negroes should turn up in 10 per cent of the inhabitants of Accra in Ghana On the other hand no Hb C is seen in East Africa nor is it found in the Belgian Congo Occasionally examples have been seen in South Africa or in Central Africa

but there is always a readily traced connection with West Africa Within that part of the continent again fairly sharp geographic demarcations are noted Westwards from Ghana the incidence falls until it becomes very low in Liberia, and there is a similar decline eastwards, where in the Yoruba in western Nigeria the incidence is only about half that found in the costal region of Ghana This was even more impressive when it was seen that within Nigeria the River Niger forms a barrier to the spread of

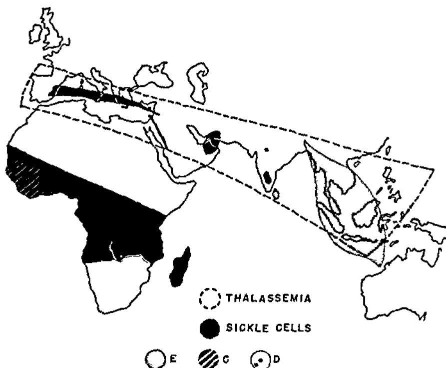



Fig 34 27 World distribution of major hemoglobin abnormalities

the hemoglobin westwards in the Yoruba Hb C is found in 5 to 6 per cent of the population east of the river in the Ibo the incidence amounts to only a fraction of 1 per cent In Ghana itself the frequency of the Hb C gene increases towards the north in the northernmost territories of Ghana and in the neighboring French High Volta territory one fifth of the population possess Hb C in their red cells There is therefore a center of high frequency somewhere in the north of Ghana with a fall in frequency to the south east and west Neel once stated that never in the history of genetics with the possible exception of Ford s melanism story in the moth have geneticists and those with kindred interests been quite so close to having a ringside seat at the origin and dissemination of a new gene (Fig 34 27)

Hemoglobin E presents a similar situation Again a very high frequency is found in Burmese and their immediate neighbors but not in Indians in nearby Bengal Tibetans or Indonesians There are small foci with a high incidence in the Indonesian archipelago but such a local increase would be expected for any gene in small islands with an inbreeding population Of particular interest is that Hb F is found in the Indo Chinese but not in the Chinese and that it is therefore linked with the earlier Mongoloid populations of Southeast Asia In Malaya there is a higher

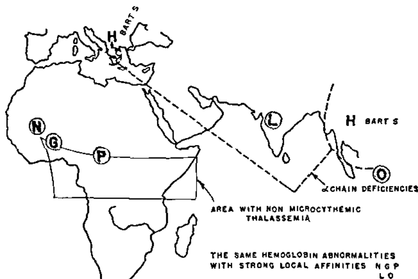



Fig 34 28 The rare hemoglobin abnormalities with strong local affinities Hb N G P L and O

incidence in the north Less hemoglobin E appears in the south where immigration from Indonesia has been of greater importance Of the primitive populations the Vedda of Ceylon have a high Hb F frequency which links them with the proto Malays rather than with the Veddoids of southern India in whom sickling but no Hb E is found This throws an entirely new light on the racial affinities of these Veddas which is actually borne out by their physical appearance Of the few proto-Malayan Pygmies examined several were Hb E trait carriers but no Hb E was discovered in the Negritos of the nearby Andaman Islands

Hemoglobin S is more widely spread It is found in tropical Africa in the west as well as in the east In the east there are striking differences between the Hamitic tribes with few if any sickle cells and the Bantus and Nilotes with high sickling rate There is evidence for a westward spread of the sickling gene and in Liberia sickling is less frequent in the

older and longer-established populations Hb S is also found in India but predominantly in primitive tribes which are the least likely to have been in contact with Africa It has been proposed that the African continent and India inherited the sickling gene independently but from a common source in the Middle East This would well agree with what is known of population movements from the Middle East towards the southeast into India

Hemoglobin D is found at high frequency in India, but it now appears that Hb $D_\alpha$ is more associated with Europeans in whom it is found occasionally, and that the Hb D itself is not uniform in India either and may be $D_\beta$ or $D_\gamma$ In India Hb D and Hb L are found in similar populations the first at high and the second at rarer frequency Although rare whenever Hb D has been discovered in Africa it has been in areas associated with the most westerly regions where it is known to occur It might be a mutation peculiar to the older inhabitants of that continent

It is remarkable that the $\alpha$ chain deficiencies Hb H and Hb Bart's should be seen in Mongoloids such as Chinese and Thais on the one hand and in Greeks on the other (Fig 34 28)

## POPULATION DYNAMICS OF THE ABNORMAL HEMOGLOBINS

It is tragic that in Africa virtually no homozygotes for the sickling gene reach adult life Many surveys particularly in East, West and Central Africa have either failed to find sickle-cell homozygotes in the adult population or have discovered them only exceptionally It is therefore surprising that the gene is present at a high frequency in many parts of that continent [160] The steady drain caused by the early death of homozygotes would be expected to cause a fall in the incidence of Hb S Frequencies of 40 per cent and more of the sickle-cell trait can be understood only if there exists a special compensating mechanism which replenishes the Hb S pool in every generation Neel [161] in 1953 discussed the implications of a situation in which the sickling gene was constantly eliminated by early death of homozygotes but in which a high frequency was nevertheless maintained One possibility is that a high mutation rate for the sickling gene is a peculiar characteristic of these populations and replaces the genes lost in every generation by sickle-cell anemia Such an explanation would require a mutation rate about three thousand times greater than those hitherto calculated for man [162] and in a search for evidence to put this theory to the test in tropical Africa no support could be found for it in an extensive survey [163] The other possibility suggested by Neel is that a balanced polymorphism exists by which the AS heterozygote enjoys two or more advantages not shared by either homozygote Allison [162] found evidence that sickle cell

trait carriers enjoy an immunity against malaria which is not shared by the normal homozygote

Several investigators have shared in developing the concept of balanced polymorphism as the genetic mechanism supporting the persistence of the sickling gene at high frequency Early observations were made in Rhodesia [164 165] It is of the greatest importance that two points be kept in mind It is necessary to single out for investigation the effect of *Plasmodium falciparum* and to separate it from the part played by other parasites because only *P falciparum* causes malignant malaria which kills children in infancy In these children it is necessary to consider only the age at which immunity against the parasite had not yet been acquired Immunity is acquired very early in hyperendemic areas and studies of adults or even of children above the age of 2 include many individuals with partial protection against *P falciparum* some of whom would be sickle-cell trait carriers and normal homozygotes [166] The protection against malaria afforded to sickling infants could by itself fully explain the high sickling rate in some African populations The mechanism of protection might be a failure of *P falciparum* to thrive on sickle-cell hemoglobin or it might be related to the fact that cells infected with this parasite tend to adhere to the vessel wall where they would become deoxygenated This would cause them to alter their shape and the sickled cells would then be phagocytosed [167] The latter is the more probable explanation because cells containing Hb S do not differ from normal cells when they are used for culture of *P falciparum* in vitro [168] Survival of homozygotes plays little part in maintaining the sickling gene at least in some parts of Africa [169 170] The protection in infancy of the heterozygotes against malignant malaria is not only the first but also the most striking example for balanced polymorphism acting in the natural selection of the human race

There is a remarkable correlation between high sickling rate and low social status [171] The lower the social level of a human group usually the greater is its isolation and inbreeding populations are notoriously likely to accumulate specific characters at a higher frequency than societies with a larger gene pool There is yet another factor which would tend to raise the sickling frequency in the more primitive tribes in the less accessible parts of Africa Although the malarial pressure may not be greater the malarial death rate will be increased with unfavorable living conditions [172] This would explain why the sickling rate is higher in some areas of Africa where the intensity of malarial infection is less than in others with a greater incidence of the sickle-cell trait With an over all increase in the death rate any differences due to protection against malaria will become more significant This suggests that the improvement of living conditions as well as the eradication of malaria will cause a fall in the sickling incidence in future generations

It has not been possible to find a difference in malarial infection between normal homozygotes and carriers of the Hb C trait [173] It has been suggested that Hb I might be responsible in part for the 'inborn' protection of infants against malaria, as for Hb E and D it has been pointed out that homozygotes who would on the whole be viable and are known to produce children would be protected against thalassemia [174] Thalassemia acts by interference with the production of Hb A and should therefore have no effect on homozygotes for Hb E or D In heterozygotes the only conditions which could arise would be Hb I thalassemia and Hb D thalassemia respectively These hemoglobinopathies are much less severe than thalassemia major It is noteworthy that both Hb E and D are found in parts of the world where it is now known that the incidence of thalassemia is high and where thalassemia major is the only hemoglobin disease which will cause the death of the affected persons with near certainty before they are able to produce children Other advantages of Hb E and D might be related to the red cell morphology of the homozygous conditions The blood picture resembles that of patients with a compensated iron deficiency There is a polycythemia without a rise in total red cell volume and perhaps such individuals can adapt themselves more easily to an iron deficient diet or to secondary iron deficiency in hookworm disease [143 175]

## THALASSEMIA

Thalassemia is an erythroblastic anemia beginning early in life which in its severe form is associated with enlargement of spleen and liver a leukemoid blood picture and characteristic bone changes Throughout the last 70 years the concept of this disease has undergone continuous change It is now known that the condition described by von Jaksch in 1889 which he called anemia pseudoleukaemica infantum and which was latter named *Jaksch Hayem Luzet anemia* included besides thalassemia such different conditions as iron deficiency anemia complicated by bacterial infection and the anemia associated with splenomegaly and cirrhosis (Banti's syndrome)

In 1925 Cooley and Lee [176] described the severe form now known as thalassemia major The name *thalassemia* or Mediterranean anemia (θαλασσα = Mediterranean Sea) was chosen [177] because it seemed at first that the disorder was found exclusively in families of Mediterranean stock Contribution from Italy and Greece [178–183] prepared the ground for the recognition that there are two similar familial conditions which vary in severity and that the major disability is found in the homozygous state The milder condition was also described in the United States where it was named *target cell anemia* and *familial microcytic anemia* [184–186]

Here also severity seemed less when the gene responsible for the abnormality was inherited as a single dominant trait [184, 185]

The key investigation which conclusively proved the mode of inheritance was that by Valentine and Neel [187, 188] this study was complemented by that of Silvestroni, Bianco and Vallisneri in Italy [189, 190]

## CLINICAL AND GENETIC DEFINITIONS

There are two ways of using the terms *thalassemia major* and *minor*. They are often employed to describe the severe and mild forms of the disease respectively. The term *thalassemia intermedia* has been introduced to describe disease states which are midway in clinical severity between thalassemia major and minor and thalassemia minima has been used when the signs and symptoms are less than in thalassemia minor

When it was thought that all homozygotes suffer from severe thalassemia and all heterozygotes from mild thalassemia the clinical and genetic interpretations of these terms were taken to be interchangeable (Fig 34 29) Although this is permissible for most cases of thalassemia there are a significant number of heterozygotes who suffer from a hemoglobinopathy which can hardly be considered a minor type. Some of these have later been found to combine the heterozygous state for thalassemia with that for an abnormal hemoglobin but others have also been proved to be solely heterozygous for thalassemia. The explanation is that the thalassemia gene is in a sense always dominant and almost always causes some symptoms although the penetrance of the gene may vary. Also since symptoms are present already however mild they may be they may be exacerbated when additional stress is experienced. A heterozygote for the hemoglobin C gene for example suffers from no known disadvantage. In the presence of bronchopneumonia or of pregnancy the new burden imposed on the hematopoietic system will affect the patient no differently than it would a normal individual. In a thalassemia heterozygote however slight symptoms would become exaggerated because the auxiliary mechanisms of blood production have already been called into action and may now no longer be capable of the expansion required for the maintenance of a normal hemoglobin level. Numerous instances of thalassemia in heterozygotes have in fact come to notice because of chronic hemorrhage or pregnancy. It would seem more helpful to reserve the terms major and minor for the genetic types and to describe the clinical conditions as severe, mild or symptomless. This convention will be employed here

## PATHOGENESIS

In thalassemia the principal disability is failure to produce red cells with normal hemoglobin content. The hemoglobin concentration may not

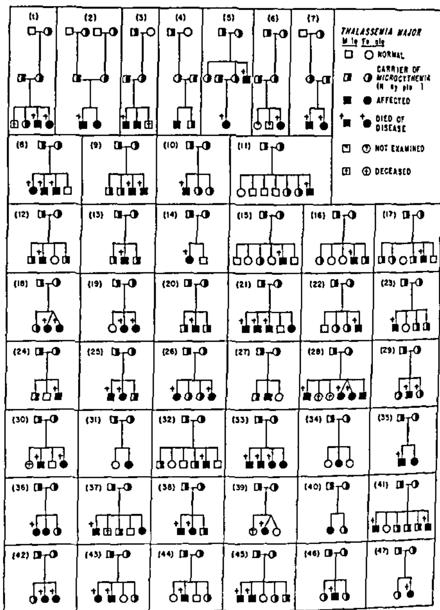



Fig 31 29 Family trees of patients suffering from thalassemia major (*By permission of M B Strauss et al [180]*)

be much below normal in these cells but they are reduced in volume The association of a low mean corpuscular volume with a normal or near normal mean corpuscular hemoglobin concentration is sometimes useful in differentiating the hypochromic anemia of thalassemia from that of iron deficiency There is a deficiency in Hb A production *[191]* because of

a disturbance in formation of ferroporphyrin [192 193] or possibly in some instances because of an abnormality in the earlier stages of porphyrin synthesis [104 105]

There is also in thalassemia increased hemolysis in the peripheral blood [196 197] By using $Fe^{59}$ to determine the sites of red cell production and to measure bone marrow activity and $Cr^{51}$ to measure red cell survival it has been demonstrated that red cell production per unit bone marrow



Fig 34 30 Appearance of the bones in thalassemia major The hypertrophy of the bone marrow causes replacement of the cortex The thinning of the cortex is compensated by numerous small trabeculae giving a mosaic pattern The metacarpals are quadrangular (*By permission of Drs A W Franklin and A R Kamp Harper St Bartholomew s Hospital London*)

is decreased but that total red cell production is greater than normal [198] Destruction of red cells is accelerated and depending upon the equilibrium which the patient establishes between increased production and increased destruction the anemia may be more or less severe At whatever level the equilibrium is finally placed it is obvious that additional blood destruction as in infectious diseases or additional demands as in pregnancy will tend to disturb the delicately balanced state

The clinical manifestations of thalassemia can all be related to the decrease in bone marrow activity per unit active tissue and to the increase in peripheral hemolysis In childhood the first observation in thalassemia is that the patient is pale and listless Intercurrent infections are poorly

resisted Splenomegaly and with lesser regularity, hepatomegaly are noted early The extension of bone marrow activity into areas of the skeleton which usually do not contain blood forming tissue and the hypertrophy of the bone marrow in those bones where hematopoiesis normally occurs affect the appearance of the skeleton There is thinning of the cortex of the long bones and the structure is supported by numerous trabeculae (Fig 34 30) X ray study discloses the mosaic pattern of this trabeculation The diploe of the skull thickens because of marrow hypertrophy and the outer and inner tables are thin Perpendicular trabeculation causes the x ray to have a 'hair-on-end' appearance The thickening of the cranial bones together with a relatively sunken nose, result in a ' mongoloid facies

APPEARANCE OF THE RED CELLS

None of the features of thalassemia is absolutely constant Of the various abnormalities changes in the red cells are found with most

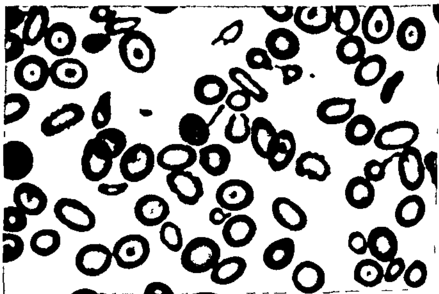


Fig 34-31 Red cells in thalassemia major Note the thinness of the cells their unequal size oval and irregularly shaped cells and target cells

regularity The overactivity of the bone marrow is reflected in the appearance of nucleated erythrocytes in the peripheral blood and in an increase in reticulocytes The red cells are thin and vary in size Abnormally shaped cells appear Since the cells are flatter than normal they tend to appear as target cells in the blood smear and can be recognized in vitro by their *increased* resistance to lysis by hypotonic salt solutions If the morphologic features of the red cells are used as the criterion for

diagnosis the reduced osmotic fragility is the most useful feature [199] The stimulation of the bone marrow often also results in an increase in white cells to give the leukemoid reaction first noted by von Jaksch (Fig 34 31)

## HEMOGLOBIN IN THALASSEMIA

Hemoglobin F is nearly always found in thalassemia major and frequently in thalassemia minor It has been doubted whether the alkali resistant hemoglobin in thalassemia is in fact the same pigment as that found in the newborn but the majority of investigators are now agreed that there are no chemical differences between the two alkali resistant hemoglobins Further rabbit serum prepared to agglutinate specifically the red cells of the newborn agglutinates also cells of patients with thalassemia [200] Hb A is increased in thalassemia from the usual 2 to 3 per cent to 3 to 5 per cent It can be assumed that both Hb A and Hb F are produced in greater quantity because the quantity of Hb $A_1$ is reduced A relation has been found between the concentration of Hb $A_2$ and individual thalassemic families [201] The amount of Hb F however varies widely and members of the same family may show widely different concentrations of this pigment Neither the level of Hb $A_2$ nor that of Hb F can be correlated in individual patients with the severity of their condition In double heterozygotes for the thalassemia gene and for a gene determining a variant of normal adult hemoglobin such as Hb S or Hb C, only the formation of Hb A seems affected Whereas in a sickle cell trait (AS) more Hb A is present than Hb S the proportion is reversed in sickle cell thalassemia

## HETEROGENEITY OF THALASSEMIA

None of the characteristics of thalassemia can be expected regularly in all cases Gouttas [202] found among 217 heterozygotes that only 99 showed the morphologic features of thalassemia as well as an increase in the Hb $A_2$ component and a persistence of fetal hemoglobin Of the remainder 109 had the typical red cell picture and an increase in Hb $A_2$ but with no Hb F Two possessed both abnormalities in hemoglobin composition but had normal red cells and two had abnormal red cells and Hb F but failed to show an increase in Hb A Two showed an increase in Hb $A_2$ as the only feature and three abnormal red cells without any hemoglobin abnormality A study of parents of patients with thalassemia major showed that the thalassemia gene may be transmitted without any signs of thalassemia [203]

There is now ample evidence that the red cell morphologic changes of thalassemia and the suppression of Hb A production can occur independently The dissociation was first observed in sickle cell heterozygotes Whereas sickle-cell thalassemia usually combines features of sickle cell

anemia with the red cell morphologic appearance of thalassemia, a number of families have now been studied in which the combination of Hb S with Hb I *and little or even no Hb A was not associated with leptocytosis*

Parents without sickle cells and children of *these individuals may* show an increased proportion of Hb I without microcytosis and in some cases without an increase in Hb $A_2$ *[204–208]* Microcytosis without Hb I has been described as a new type of hemoglobinopathy *[210]* or as 'anemia hypochromica sideroblastica hereditaria' *[209]* The finding of Hb I without microcytosis has been termed variously 'nonmicrocythemic thalassemia' 'hereditary persistence of fetal hemoglobin' or the 'I gene condition Gerald *[148]* has gone so far as to postulate a "new" hemoglobinopathy for any thalassemic condition found without an increase in Hb $A_2$

There is no doubt that the dissociation of the features of thalassemia is most easily explained on the basis of a multiplicity of genes which are usually inherited together but which can also be transmitted independently The inheritance of one 'nonmicrocythemic' and one 'classical thalassemia gene results in typical thalassemia major *[211]* as does that of the Lepore trait together with a single gene for classical thalassemia *[201]* *Hemoglobin H disease is now recognized as being allied to* thalassemia and as requiring the inheritance of at least one thalassemia gene Yet in this condition Hb $A_2$ is reduced rather than increased On the other hand, no noticeable deficiency in Hb $A_2$ has been noted in thalassemic children with Hb Bart's This is surprising because both Hb H and Hb Bart s are $\alpha$ chain deficiencies one presenting as an excess of the $\beta$ chain of adult hemoglobin, and the other as a tetramer of the fetal $\gamma$ chain In two patients with $\beta$ chain deficiency in both fetal and adult hemoglobin resulting in both Hb H and Bart s, Hb $A_2$ was reduced as in Hb H disease

The multiplicity of the thalassemia genes is borne out further by family studies which have demonstrated that in some families the thalassemia gene is inherited independently of the sickling gene and in others in a *manner* which suggests either allelism or close linkage *[212, 213]* There can be little doubt but that the early suggestions that there might exist several thalassemia genes *[8 214 215]* heralded a picture of much complexity The diagnosis of thalassemia is today a collective concept no less than was the von Jaksch Hayem Luzet syndrome two or three generations ago

## GEOGRAPHIC DISTRIBUTION OF THALASSEMIA

Most of the early work on thalassemia was on families of Mediterranean origin *There is now no doubt that the term is a misnomer* and that thalassemia is most commonly found in Asia Cases observed in Turks Persians Kurdistan Jews *[216–219]* and even those seen in India *[220]*

might be related to cases among the Mediterranean populations but this explanation fails to account for thalassemia in Thailand [221] Although no survey has yet been made in China Chernoff [222] lists eight accounts of thalassemia in Chinese most all of whom originated from the area around Canton and Hongkong One of the authors of this chapter (H L ) has seen thalassemia in eight Chinese families in London England Numerous observations have been made in Indonesia [223] and the Philippines [224]

## POPULATION DYNAMICS OF THALASSEMIA

The homozygote for thalassemia suffers from a severe disease and only rarely survives into adult life Those who do are sexually immature Yet in Italy alone the number of thalassemic individuals has been estimated to surpass 1 000 000 A world incidence of many tens of millions would not be an exaggerated estimate

The high frequency of a gene which for all practical purposes cannot be transmitted by the homozygote and which may cause disease even in the heterozygote requires an explanation It has been suggested that parents of children who died of thalassemia major might tend to over compensate their loss Since both parents would be heterozygous for thalassemia a greater fecundity from such unions would result in an increase in the number of heterozygotes in the following generation The theory of overcompensation has been carefully tested in Italy but the available evidence failed to support it [225]

Haldane [175] has made an alternative suggestion that a balanced polymorphism might favor the survival of the thalassemia heterozygote He suggested that while the abnormal homozygote dies from the anemia the normal homozygote might be less capable than the heterozygote of adjusting his blood formation to an iron or vitamin deficiency How such a protection against iron deficiency in the heterozygote might come about has been described by Sijpesteijn [226] Women with thalassemia minor tend to have a mild hypochromic anemia which causes them to increase iron absorption Since in thalassemia iron utilization for hemoglobin production is decreased these women would store their iron and eventually would have larger deposits of this element than normal women They would then be better protected against the iron deficiency anemia of multiparous women which so often is the cause of death in this group in primitive populations They would also presumably have an advantage when ancylostomiasis causes chronic hemorrhage and subsequent iron deficiency Haldane also suggested that thalassemia hetero zygotes might be better protected against malaria than normal homozygotes and some evidence for this theory has been found in Sardinia [illegible] Of two racially identical populations one lived in a highly malarious region and also showed a high frequency of the thalassemia gene and

the other lived in a healthier area and showed a much lower incidence of this abnormality

Whatever the genetic advantage of the thalassemia gene has been in the past or is today in underdeveloped countries the inheritance of thalassemia is now a major problem in some advanced countries where infantile mortality due to other causes such as infection and malnutrition has fallen Marriage advice has been widely advocated in Italy The results of the work on bone marrow transfusion and transplantation undertaken in conjunction with leukemia and radiation damage will have to be followed with the greatest attention by those interested in the thalassemia problem and indeed that of the hemoglobinopathies as a whole One might plan such transplantations for the newborn when antibody formation is still undeveloped Perhaps the day will come when every child of two parents with the thalassemia trait (or the sickle-cell trait) will receive a bone marrow transplantation from either or both parents at birth without any delay for the diagnosis of the infant s own hemoglobin genotype

## SUMMARY

1 The hemoglobins of man are a group of pigments each of which is composed of four molecules of ferroprotoporphyrin IX attached to one molecule of globin The hemoglobins differ in the properties of the globin moiety

2 At birth fetal hemoglobin Hb F predominates In normal man this is rapidly replaced by the normal adult pigment Hb A Hb A consists of at least two components a major one $A_1$ and a minor component Hb $A_2$

3 The globin molecule consists of two identical half molecules each of which contains two different long peptide chains In Hb A these are the $\alpha$ and $\beta$ chains and in Hb F the $\alpha$ and the $\gamma$ chains The variant hemoglobins contain either altered combinations of the four chains or altered amino acid sequence within the chains Each type of chain is thought to correspond to one gene and the inheritance of the polypeptide chains is codominant

4 Abnormal hemoglobins are principally identified by their electrophoretic properties The most important abnormal hemoglobin is sickle-cell hemoglobin Hb S which is responsible for the sickling phenomenon in sickle cell disease Reduced solubility of Hb S under reduced oxygen tension is responsible for the various clinical and laboratory phenomena of the disease Heterozygotes for Hb S and Hb A are not anemic but heterozygotes for Hb S and for other abnormal hemoglobins or for thalassemia may be anemic if Hb S becomes the predominant hemoglobin

5 Almost all hemoglobins contain $\alpha$ chains The exceptions are Hb H which has four $\beta$ chains and Hb Bart s which has four $\gamma$ chains Of the

α chain hemoglobins only one Hb F which is $\alpha_2\gamma_2$ contains no β chains The other α chain hemoglobins are normal hemoglobin those with an α chain mutational change (Hb D I P and Q) and those with a β chain mutational change (Hb S C, $D_\beta$ E G J L and N) The properties and clinical significance of these and other less well characterized hemoglobins are described in the text

6 The world distribution of the variant hemoglobins has provided important anthropologic data and has contributed to the study of population dynamics In the case of Hb S the high frequency of trait carriers is thought to derive from a selective advantage of Hb AS heterozygotes in areas with high mortality from *P falciparum*

7 Thalassemia a familial microcytic anemia is not known at present to be the result of an abnormality of hemoglobin structure but rather may be an abnormality in the rate of synthesis of Hb A The inheritance pattern in the majority of families studied is codominant with and segregating independently of abnormal hemoglobin traits Thalassemia and abnormal hemoglobins may coexist and their combination is often manifested by anemia

## BIBLIOGRAPHY


1 von Korber E Über Differenzen des Blutfarbstoffes Dissertation Dorpat 1886 Zentralbl med Wiss 177 1867

2 Jonxis J H P Foetal haemoglobin in children in *Abnormal Haemoglobins* p 114 Blackwell Oxford 1959

3 Bianco I La Resistenza emoglobinica nei portatori di microcithemia e di falcemia Policlinico (sez prat) 55 103 1948

4 Lutignano T and Fiore Donati L La Resistenza emoglobinica nel M di Cooley (del bambino e dell adulto) e forme affini Bull soc ital biol sper 24 77 1948

5 Vecchio F Sulla resistenza dell emoglobinica alla denaturazione alcalina negli ammalati di anemia di Cooley e nei loro familiari Prog med Napoli 4 201 1948

6 Liquori A M Presence of foetal haemoglobin in Cooley s anaemia Nature London 167 950 1951

Singer K Chernoff A I and Singer L Studies on abnormal hemoglobin Blood 6 413 1951

8 White J C and Beaven G H Review of varieties of human haemoglobin in health and disease J Clin Path 7 175 1954

9 Betke K Der menschliche Blutfarbstoff bei Fetus und reifem Organismus Springer Berlin and Vienna 1954

10 Derrien Y On the heterogeneity of normal human hemoglobin in *Abnormal Haemoglobins* p 63 Blackwell Oxford 1959

11 Itano H A Solubilities of naturally occurring mixtures of human hemoglobin Arch Biochem 47 148 1953

1 Kunkel H G and Wallenius G New hemoglobin in normal adult blood Science 122 288 1955

13 Ceppellini R Kunkel H G and Dunn L *Symposium on the Study of Human Variation* Columbia University Press New York 1958

14 Ma n M S Josephson A M and Singer I Starch block electrophoretic studies of human hemoglobin solution Blood 13 533 1958

15 H rl in H and Weber G Über chronische familiäre Methämoglobinämie und eine neue Modifikation des Methämoglobin Deutsche med Wchnschr 73 4 1948

16 Watson J The significance of the paucity of sickle cells in newborn negro infants Am J M Sc 216 419 1948
17 Pauling L Itano H A Singer S J and Wells I C Sickle cell anemia a molecular disease Science 110 543 1949
18 Herrick J B Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia Arch Int Med 6 517 1910
19 Hahn E V and Gillespie E B Sickle cell anemia Arch Int Med 39 233 1927
20 Diggs L W Ahmann C F and Bibb J The incidence and significance of the sickle cell trait Ann Int Med 7 769 1933
21 Sherman I J The sickling phenomenon with special reference to the differentiation of sickle cell anemia from the sickle cell trait Bull Johns Hopkins Hosp 67 309 1940
22 Singer K Robin S King J C and Jefferson R N The life span of the sickle cell and the pathogenesis of sickle cell anemia J Lab Clin Med 33 975 1948
23 Callender S T E Nickel J F Moore C V and Powell E O Sickle cell disease studied by measuring the survival of transfused red blood cells J Lab Clin Med 34 90 1949
24 Emmel V E A study of the erythrocytes in a case of severe anemia with elongated and sickle shaped red blood corpuscles Arch Int Med 20 586 1917
25 Taliaferro W H and Huck J G The inheritance of sickle-cell anemia in man Genetics 8 594 1923
26 Neel J V The clinical detection of genetic carriers of inherited disease Medicine 26 115 1947
27 Beet E A Genetics of sickle-cell trait in a Bantu tribe Ann Eugenics 14 279 1949
28 Neel J V The inheritance of sickle cell anemia Science 110 64 1949
29 Harris J W Studies on the destruction of red blood cells VIII Molecular orientation in sickle cell hemoglobin solutions Proc Soc Exper Biol & Med 75 197 1950
30 Perutz M F and Mitchison J M State of haemoglobin in sickle-cell anemia Nature London 166 677 1950
31 Perutz M F and Liquori A M X ray and solubility studies of the haemoglobins of sickle-cell anemia patients Nature London 167 929 1951
32 Greenberg M S Kass E H and Castle W B Studies on the destruction of red blood cells XII Factors influencing the role of hemoglobin in the pathologic physiology of sickle cell anemia and related disorders J Clin Invest 36 833 1957
33 Harris J W Brewster H H Ham T H and Castle W B Studies on destruction of red blood cells biophysics and biology of sickle-cell disease A M A Arch Int Med 97, 145 1956
34 Allison A C Observations on the sickling phenomenon and on the distribution of different haemoglobin types in erythrocyte populations Clin Sc 15 497 1956
35 Goodwin W E Alston E F and Semans J H Hematuria and sickle cell disease unexplained gross unilateral renal hematuria in Negroes coincident with the blood sickling trait J Urol 63 79 1950
36 Chernoff A I The human hemoglobins in health and disease New England J Med 253 322 365 416 1955
37 Neel J V Kaplan E Zuelzer W W Further studies on Hemoglobin C I A description of 3 additional families segregating for Hemoglobin C and sickle cell hemoglobin Blood 8 724 1953
38 Itano H A and Neel J V A new inherited abnormality of human hemoglobin Proc Nat Acad Sc 36 613 1950
39 Ranney H M Larson D L and McCormack G H Jr Some clinical biochemical and genetic observations on hemoglobin C J Clin Invest 32 1 77 1953
40 Edington G M and Lehmann H A case of sickle-cell haemoglobin C disease and a survey of haemoglobin C incidence in West Africa Tr Roy Soc Trop Med & Hyg 48 332 1954

41 Scheinberg I H Harris R S and Spitzer J L Differential titration by means of paper electrophoresis and the structure of human hemoglobins Proc Nat Acad Sc 40 777 1954

42 Scheinberg I H The structural basis of differences in electrophoretic behavior of human hemoglobins in *Conference on Hemoglobin* p 2 7 National Academy of Sciences National Research Council Publication 557 Washington 1958

43 Kendrew J C and Perutz M F X ray studies of compounds of biological interest Ann Rev Biochem 26 327 1957

44 Cullis A F Dintzis H M and Perutz M F X ray analysis of haemoglobin in *Conference on Hemoglobin* p 50 National Academy of Sciences National Research Council Publication 557 Washington 1958

45 Rhinesmith H S Schroeder W A and Pauling L The N terminal amino acid residues of normal adult hemoglobin a quantitative study of certain aspects of Sanger s DNP method J Am Chem Soc 79 609 1957

46 Rhinesmith H S Schroeder W A and Martin N The N terminal sequence of the β chains of normal adult human hemoglobin J Am Chem Soc 80 3358 1958

47 Ingram V M A specific chemical difference between globins of normal human and sickle-cell anaemia haemoglobin Nature London 178 79 1956

48 Ingram V M Gene mutations in human haemoglobin the chemical difference between normal and sickle cell haemoglobin Nature London 180 3 6 195

49 Ingram V M Abnormal human haemoglobins I The comparison of normal human and sickle-cell haemoglobins by fingerprinting Biochim et biophys acta 28 539 1958

50 Hunt J A and Ingram V M Allelomorphism and the chemical differences of the human haemoglobins A S and C Nature London 184 1062 1958

51 Itano H A A third abnormal hemoglobin associated with hereditary hemolytic anemia Proc Nat Acad Sc 37 775 1951

52 Benzer S Ingram V M and Lehmann H Three varieties of human haemoglobin D Nature London 182 853 1958

53 Ingram V M Chemistry of the abnormal human haemoglobins Brit M Bull 15 27 1959

54 Murayama M and Ingram V M Comparison of normal adult human haemoglobin with haemoglobin I by fingerprinting Nature London 183 1798 1958

55 Hunt J A and Ingram V M A terminal peptide sequence of human haemoglobin Nature London 184 640 1959

56 Singer S J and Itano H A On the asymmetrical dissociation of human hemoglobin Proc Nat Acad Sc 45 174 1959

57 Vinograd J R Hutchison W D and Schroeder W A C hybrids of human hemoglobins II The identification of the aberrant chain in human hemoglobin S J Am Chem Soc 81 3168 1959

58 Itano H A and Robinson E Formation of normal and doubly abnormal haemoglobins by recombination of haemoglobin I with S and C Nature London 183 1799 1959

59 Itano H A and Robinson E Properties and inheritance of hemoglobin by asymmetric recombination Nature London 184 1468 1959

60 Neel J V Genetic aspects of human hemoglobins in *Conference on Hemoglobin* p 253 National Academy of Sciences National Research Council Publication 557 Washington 1958

61 Smith E W and Torbert J V Study of two abnormal hemoglobins with evidence for a new genetic locus for hemoglobin formation Bull Johns Hopkins Hosp 102 38 1958

62 Cabannes R and Portier A Paper electrophoresis examination of newly discovered hemoglobins in *Abnormal Haemoglobins* p 51 Blackwell Oxford 1959

63 Dherte P Lehmann H and Vandepitte J Haemoglobin P in a family in the Belgian Congo Nature London 184 1133 1959

64 Lambotte Legrand J Lambotte-Legrand C Ager J A M and Lehmann H Rev hémat (in press)

65 Schwartz H C Spaet T H Zuelzer W W Neel J V Robinson A R and

16 Watson J The significance of the paucity of sickle cells in newborn negro infants Am J M Sc 215 419 1948
17 Pauling L Itano H A Singer S J and Wells I C Sickle cell anemia a molecular disease Science 110 543 1949
18 Herrick J B Peculiar elongated and sickle-shaped *red blood corpuscles in a* case of severe anemia Arch Int Med 6 517 1910
19 Hahn E V and Gillespie E B Sickle cell anemia Arch Int Med 39 233 1927
20 Diggs L W Ahmann C F and Bibb J The incidence and significance of the sickle cell trait Ann Int Med 7 769 1933
21 Sherman I J *The sickling phenomenon with special reference to the differentia* tion of sickle cell anemia from the sickle cell trait Bull Johns Hopkins Hosp 67 309 1940
22 *Singer K Robin S King J C and Jefferson R N The life span of the sickle* cell and the pathogenesis of sickle cell anemia J Lab Clin Med 33 975 1948
23 Callender S T E Nickel J F Moore C V and Powell E O Sickle cell *disease studied by measuring the survival of transfused red blood cells J Lab* Clin Med 34 90 1949
24 Emmel V E A study of the erythrocytes in a case of severe anemia with elon*gated and sickle-shaped red blood corpuscles Arch Int Med 20 586 1917*
25 Taliaferro W H and Huck J G The inheritance of sickle-cell anemia in man Genetics 8 594 1923
26 Neel J V The clinical detection of genetic carriers of inherited disease Medicine 26 115 1947
27 Beet E A Genetics of sickle-cell trait in a Bantu tribe Ann Eugenics 14 279 *1949*
28 Neel J V The inheritance of sickle cell anemia Science 110 64 1949
29 Harris J W Studies on the destruction of red blood cells VIII Molecular orientation in sickle cell hemoglobin solutions Proc Soc Exper *Biol & Med* 75 197 1950
30 Perutz M F and Mitchison J M State of haemoglobin in sickle-cell anemia Nature London 166 677 1950
31 Perutz M F and Liquori A M X ray and solubility studies of the haemoglobins of sickle-cell anemia patients Nature London 167 929 1951
32 Greenberg M S Kass E H and Castle W B Studies on the destruction of red blood cells XII Factors influencing the role of hemoglobin in the pathologic physiology of sickle cell anemia and related disorders J Clin Invest 36 833 1957
33 Harris J W Brewster H H Ham T H and Castle W B Studies on destruction of red blood cells biophysics and biology of sickle-cell disease A M A Arch Int Med 97 145 1956
34 Allison A C Observations on the sickling phenomenon and on the distribution of different haemoglobin types in erythrocyte populations Clin Sc 15 497 1956
35 Goodwin W E Alston E F and Semans J H *Hematuria and sickle cell* disease unexplained gross unilateral renal hematuria in Negroes coincident with the blood sickling trait J Urol 63 79 1950
36 Chernoff A I The human hemoglobins in health and disease *New England J* Med 253 322 365 416 1955
37 Neel J V Kaplan E Zuelzer W W Further studies on Hemoglobin C I A description of 3 additional families segregating for *Hemoglobin C and sickle cell* hemoglobin Blood 8 724 1953
38 Itano H A and Neel J V A new inherited abnormality of human hemoglobin Proc Nat Acad Sc 36 613 1950
39 Ranney H M Larson D L and McCormack G H Jr Some clinical biochemical and genetic observations on hemoglobin C J Clin Invest 32 1277 1953
40 Edington C M and Lehmann H A case of sickle-cell haemoglobin C disease and a survey of haemoglobin C incidence in West Africa Tr Roy Soc *Trop* Med & Hyg *48 332 1954*

92 Fe sas P Ma trokalo N Fostiropoulo ( Vella F Ag r J A M and Lehmann H New varient of l uman foetal h iemoglobin Nature Lon lon 183 30 1959

93 Biaven G H and ( ratzer W B A critical review of human haemoglobin variants I Methods for separation and cl aracterization J Clin I ath 12 1 1J 9

94 Juisman T H and I rins H K The chromatographic behaviour of different abnormal human haemoglobins on the cation exchanger amberlite IRC 50 Clin chim acta 2 307 1957

95 Fessas P and Papaspyrou A New fast hemoglobin a ociated with thalassemia Science 126 1119 1957

96 Ogden A L Lehmann H and Rimington C D cussion on inherited metabolic characters in man and animals I roc Roy Soc Me l 52 555 1959

97 Allison A C Notation for hemoglobin types and genes controlling their synthesis Science 122 640 1955

98 Allison A C Personal communication

99 Halbrecht I and Klibanski C Identification of a new normal embryonic haemoglobin Nature London 178 794 1956

100 Halbrecht I Klibanski C and Bar Ilan F Co-existence of the embryonic (third normal) haemoglobin fraction with erytl roblastosis in the blood of two full term new born babies with multiple malforn at on Nature London 183 3 1359

101 Drescher H and Kunzer W Der Blutfarbstoff des ı cr chl chen Feten Klin Wehnschr 32 J 1954

1) Kunzer W and Drescher H Spektrophotometrische Untersuchungen am Blutfarbstoff menschlicher Feten Klin Wel nschr 34 918 1956

103 Kunzer W Human embryo haemoglobins Nature London 179 477 1957

104 Beaven G H Hoch H and Holiday E R Tl e haemoglobins of the human fetus and infant Electrophoretic and spectroscopi differentiation of adult and foetal types Biochem J 49 374 1951

105 Walker J and Turnbull E P N Haemoglobin and red cells in the human foetus III Foetal and adult l aemoglobin Arch Dis Childhood 30 111 1955

106 Jones R T Blood (in press)

10 Vella F Heterogeneity of human foetal haemoglobin incidence of foetal var an s in Singapore Nature London 184 2,? 1959

108 Morrison M and Cook J L Chromatograpl ic fractionatio of normal adult oxyhemoglobin Science 122 9 0 1955

109 Huisman T H J and Prins H K Chromatographi estimation of 4 different human hemoglobins J Lab & Clin Med 46 55 1955

110 Allen D W Schroeder W A and Balog J Ol er t ons on tl e cl romatographic heterogeneity of normal adult and f tal l uman l cmoglobin a study of the effects of crystallization and cl romatography on the l eterogeneity and isoleucine content J Am Chem Soc 80 16 8 1J 8

111 Fessas P Personal communication

112 Choremis C Zannos Manolea L Ager J A M and Lel mann H Persistence of haemoglobin Bart s beyo d infa cy in a cl ild with tl alassemia Brit M J 2 348 19 9

113 Lng L I L Haemoglobin of new born infants in Indonesia Nature London 183 11 5 1959

114 Hend icksc R G Brit M J (in pres )

115 Huisman T H J Tl e identifi at on of l uman haemoglobins in *Abnormal Ha mogl bi* p 18 Blackv ell Oxford 1 ) J

116 Tuchinda S Vareenil C Bhanclit I n l Minnich V Fast hemoglobin component foun l in uml il cal-cord blood of Thai babi I iatric 24 43 1959

117 Ag r J A M Hunt J A Koller H an l I l mann H I roceed ng of th Congr ss of I urope in Society of Haet atolc gy 1J )) (1J(0) (in pres )

118 S l n ıler R G I rsonal communi ation

119 Fessas I Haemoglobin Bart s Brit M J 2 880 1959

Kaufman S F Combinations of hemoglobin G hemoglobin S and thalassemia occurring in one family Blood 12 238 1957
66 Edington G M and Lehmann H Haemoglobin G A new haemoglobin found in a West African Lancet 2 173 1954
67 Hill R L and Schwartz H C A chemical abnormality in Haemoglobin G Nature London 184 641 1959
68 Motulsky A G Genetic and haematological significance of Haemoglobin H Nature London 178 1055 1956
69 Jones R T Schroeder W A Balog J E and Vinograd J H Gross structure of hemoglobin H J Am Chem Soc 81 3161 1959
70 Schroeder W A and Matsuda G N terminal residues of human fetal hemoglobin J Am Chem Soc 80 1521 1958
71 Hunt J A Identity of the α-chains of adult and foetal human haemoglobins Nature London 183 1373 1959
72 Katz A M and Chernoff A I Structural similarities between hemoglobins A and J Science 130 1574 1959
73 Ager J A M and Lehmann H Observations on some fast haemoglobins K J N and Bart s Brit M J 1 929 1958
74 Hunt J A and Lehmann H Haemoglobin Bart s a foetal haemoglobin without α-chains Nature London 184 87 1959
75 Ramot B Sheba C Fisher S Ager J A M and Lehmann H Haemoglobin H disease with persistent haemoglobin Bart s in an Oriental Jewess and her daughter Brit M J 2 1228 1959
76 Chernoff A I Fisher B Harris J W Itano H A Kaplan E Singer K and Neel J V A system of nomenclature for the varieties of human hemoglobin Science 118 116 1953
77 Singer K Hereditary hemolytic disorders associated with abnormal hemoglobins Am J Med 18 633 1955
78 Ceppelini R L emoglobina normale lenta $A_2$ Symposium geneticae haematologicae Acta genet med et gemel 8 Suppl II p 47 1959
79 Cabannes R and Buhr L Une nouvelle fraction hémoglobinique humaine à migration électrophorétique plus rapide que l hémoglobine A et moins rapide que l hémoglobine H note préliminaire Pédiatrie 10 858 1955
80 Rucknagel D L Page E B and Jensen W N Hemoglobin I an inherited hemoglobin anomaly Blood 10 999 1955
81 Jensen W N Page E B and Rucknagel D L Hemoglobin H an inherited hemoglobin anomaly Clin Res Proc 3 93 1955
82 Rigas D A Koler R D and Osgood E E New hemoglobin possessing a higher electrophoretic mobility than normal adult hemoglobin Science 121 372 1955
83 Cabannes R Duzer A Portier A Massonnat J and Sendra L Hémoglobines anormales chez l algérien musulman bilan statistique de deux années d études portant sur 1 877 hémoglobinogrammes Sang 27 580 1956
84 Thorup O A Itano H A Wheby M and Leavell B S Hemoglobin J Science 123 889 1956
85 Lehmann H The hemoglobinopathies Blood 12 90 1957
86 Robinson A R Zuelzer W W Neel J V Livingstone F B and Miller M Two fast hemoglobin components in Liberian blood samples Blood 11 902 1956
87 Schneider R G and Haggard M E A new haemoglobin variant exhibiting anomalous electrophoretic behaviour Nature London 180 1486 1957
88 Eng L I L A new haemoglobin in the Buginese Lancet 2 1338 1957
89 Ager J A M Lehmann H and Vella F Haemoglobin Norfolk a new haemoglobin found in an English family Brit M J 2 539 1958
90 Albahary C Dreyfus J C Labie D Schapira G and Tram L Hémoglobines anormales au Sud Vietnam hémoglobinose C homozygote trait E hémoglobine nouvelle Rev hémat 13 163 1958
91 Dherte P Vandepitte J Ager J A M and Lehmann H Stanleyville I and II Brit M J 2 282 1959

143 Lehmann H Di tribution of abnormal haemoglobins J Clin Path 9 180 1956
144 Itano H A Bergren W R and Sturgeon P Identification of a fourth abnormal human hemoglobin J Am Chem Soc 76 2 ι8 1954
145 Chernoff A I Minnich V and Chongchareonsuk S Hemoglobin E a hereditary abnormality of human hemoglobin Science 120 605 1954
146 Aksoy H and Lehmann H The first ob ervation of sickle-cell haemoglobin E disease Nature London 179 1?48 19ο7
147 Hunt J A and Ingram V M Human haemoglobin E the chemical effect of gene mutation Nature London 184 8 0 19ο9
148 Gerald P S The electrophoretic and spectroscopic characterization of Hgb M Blood 13 936 1958
149 Gouttas A Fessas P Tsevrenis H and Xefteri E Description d une nouvelle variété d anémie hémolytique congénitale (étude hématologique électrophoretique et génétique) Sang 26 911 19ὸο
150 Gerald P S and Diamond L K A new hereditary hemoglobinopathy (the Lepore trait) and its interaction with thalassemia trait Blood 13 83 1958
151 Vella F Ager J A M and Lehmann H An abnormal haemoglobin in a Chinese haemoglobin G Nature London 182 460 1958
152 Ager J A M and Lehmann H Haemoglobin L a new haemoglobin found in a Punjabi Hindu Brit M J 2 142 1957
153 Sukumaran P K Sanghvi L D Ager J A M and Lehmann H Haemoglobin L in Bombay Acta genet 9 ?0? 1959
154 Vella F and Singh R B Haemoglobin L in Indians in Malaya Tr Roy Soc Trop Med & Hyg 53 534 1959
155 Schroeder W A and Jones R T Personal communication
156 Eng L I L and Sadono Haemoglobin O (Buginese X) in Sulawesi Brit M J 1 1461 1958
15 Schneider R G and Haggard M E Haemoglobin P (the Galveston type) Nature London 182 3? 1958
158 Vella F Wells R H C Ager J A M and Lehmann H A haemoglobinopathy involving haemoglobin H and a new (Q) haemoglobin Brit M J 1 75? 1958
159 Lehmann H Distributions of variations in human haemoglobin synthesis in *Abnormal Haemoglobins* p 20? Blackwell Oxford 1959
160 Lehmann H The maintenance of the haemoglobinopathies at high frequency A consideration of the relation between sickling and malaria and of allied problems in *Abnormal Haemoglobins* p 307 Blackwell Oxford 1959
161 Neel J V Data pertaining to population dynamics of sickle cell disease Am J Human Genet 5 154 1953
16 Allison A C Protection afforded by sickle-cell trait against subtertian malarial infection Brit M J 1 ?0 1954
163 Vandepitte J M Zuelzer W W Neel J V and Colaert J Evidence concerning the inadequacy of mutation as an explanation of the frequency of the sickle cell gene in the Belgian Congo Blood 10 341 19ο5
164 Beet E A Sickle cell disease in the Balovale district of Northern Rhodesia East African M J 23 5 1956
165 Brain P Sickle cell trait its clinical significance South African M J 26 9? 1952
166 Raper A B Malaria and the sickling trait Brit M J 1 118? 1955
167 Miller M J Neel J V and Livingstone F B Distribution of parasites in the red cells of sickle-cell trait carriers infected with *Plasmodium falciparum* Tr Roy Soc Trop Med & Hyg 50 9 4 1956
168 Raper A B Further observations on sickling and malaria Tr Roy Soc Trop Med & Hyg 53 110 1959
169 Lehmann H and Raper A B Maintenance of high sickling rate in an African community Brit M J 2 333 1956
170 Allison A C Sickle cell and haemoglobin C genes in some African populations Ann Human Genet 21 67 1956

120 Ingram V M and Stretton A O W Genetic basis of the thalassaemia diseases Nature London 184 1903 1959
121 Ldington G M Lehmann H and Schneider R G Characterization and genetics of haemoglobin G Nature London 176 850 1955
122 Castle W B and Daland G A Susceptibility of mammalian erythrocytes to hemolysis with hypotonic solutions function of differences between discoidal volume and volume of sphere of equal surface Arch Int Med 60 949 1937
123 Schwartz S O S and Hartz W H Jr Mediterranean anemia in the Negro A re-evaluation of four patients and their families Blood 10 1256, 1955
124 Silvestroni L and Bianco I Singlore associazone di anemia microcitica *costituzionale con drepanocito anemia* in saggetto di razza bianca Ioli clinico (sez prat) 53 265 1946
125 Gatto I and Iurrazzella G Ulteriori richerche di genetica sulla drepanocitosi Iediatria 59 789 1951
126 Powell W N Rodarte J G and Neel J V The occurrence in a family of Sicilian ancestry of the traits for both sickling and thalassemia Blood 5 887 1950
127 Wasserman C F Phelps V R and Hertzog A J Chronic hemolytic anemia in a white child due to thalassemia and sicklemia Iediatrics 9 286 1952
128 Sturgeon P Itano H A and Valentine W N Chronic hemolytic anemia associated with thalassemia and sickling traits Blood 7 350 1952
129 Neel J V Itano H A and Lawrence J S Two cases of sickle cell disease presumably due to the combination of the genes for thalassemia and sickle cell hemoglobin *Blood 8 434 1953*
130 Humble J G Anderson I White J C and Freeman T Family illustrating double inheritance of sickle cell trait and of Mediterranean anaemia J Clin Iath 7 201 1954
131 *Smith L W and Conley C L Clinical features of the genetic* variants of sickle cell disease Bull Johns Hopkins Hosp 94 289 1951
132 Singer K Singer L and Goldberg S R Studies on abnormal hemoglobins XI Sickle cell thalassemia disease in the negro The significance of the S + A + F and S + A patterns obtained by hemoglobin analysis Blood 10 405 1955
133 Singer K Kraus A P Singer L Rubinstein H M and Goldberg S R Studies on abnormal hemoglobins X A new syndrome hemoglobin C-thalassemia disease Blood 9 1032 1954
134 Zuelzer W W and Kaplan E *Thalassemia hemoglobin C disease A new* syndrome presumably due to the combination of the genes for thalassemia and hemoglobin C Blood 9 1047 1954
135 Minnich V Na Nakorn S Chongchareonsuk S and Kochaseni S Mediterranean anemia a study of thirty two cases in Thailand Blood 9 1 1954
136 Sturgeon I Itano H A and Bergren W R Clinical manifestations of inherited abnormal hemoglobins II Interaction of hemoglobin E and thalassemia trait Blood 10 396 1955
137 Chernoff A I Minnich V Na Nakorn S Tuchinda S Kashemsant C and Chernoff R Studies on Hemoglobin E I The clinical hematologic and genetic characteristics of the hemoglobin E syndromes J Lab & Clin Med 47 455 1956
138 Lehmann H Story P and Thein H Haemoglobin E in Burmese Two cases of haemoglobin E disease Brit M J 1 544 1956
139 Eng L I L and Giok O H Homozygous haemoglobin E disease in Indonesia Lancet 1 20 1957
✓140 Sanghvi L D Sukumaran P K and Lehmann H Haemoglobin J trait in 2 Indian women Associated with thalassemia in one Brit M J 2 828 1958
141 Itano H A A third abnormal hemoglobin associated with hereditary hemolytic anemia Proc Nat Acad Sc 37 775 1951
142 Sturgeon I Itano H A and Bergren W R Clinical manifestations of inherited abnormal hemoglobins I The interaction of hemoglobin S with hemoglobin D Blood 10 389 1955

199 S nisealco M and Smith C A B The discrimination between normals and mi rocythaemics as a statistical problem in *Compt s rendus du V congrès inter national de transf sion sanguine* p 483 Croutz t Paris 1954
200 Ikin F W Lehmann H and Hutton P W An agglutinin for the red cells of newborn infants in *Comptes rendus du V co grès international de transfusion sang ine* p 371 Croutzet Paris 1954
201 Gerald P S and Diamond L K The diagnosis of thalassemia trait by starch block electrophoresis of the hemoglobin Blood 13 61 1958
20 Gouttas A *Proce d gs ith European Congress of Hae atology* London 1959 Acta haemat (in press)
03 Zuelzer W W Neel J V and Robinson A R Abnormal hemoglobin in *Prog ess in Hematology* p 91 Grune & Stratton New York 1956
204 Edington C M and Lehmann H Expression of the sickle-cell gene in Africa Brit M J 1 1308 2 1378 1955
05 Neel J V Hiernaux J Linhard J Robinson A Zuelzer W W and Livingstone F R Data on occurrence of hemoglobin C and other abnormal hemoglobins in some African populations Am J Human Genet 8 138 1956
06 Jacob G F and Raper A B Hereditary persistence of foetal haemoglobin production and its interaction with the sickle-cell trait Brit J Haemat 4 138 19 8
07 Went L N and MacIver J E An unusual type of hemoglobinopathy resembling sickle-cell thalassaemia in a Jamaican family Blood 13 559 1958
208 Chernoff A I The thalassemia syndromes *Conf rence on Hemoglobin* p 283 National Academy of Sciences National Research Council Washington 1958
209 Caroli J Bernard J B ssis M Combrisson A Malassenet R and Breton J Hémochromatose avec anémie hypochrome et absence d hémoglobine anormale étude au microscope electronique Presse méd 65 1991 1957
210 Heilmeyer L Emmerich J Hennemann H H Kleiderling W Lee M Bilger R and Schubothe H Über eine chronische hypochrome Anämi bei zwei Geschwistern auf der Grundlage einer Eisenverwertungsstorung Anaemia hypochromica sideroblastica hereditaria Folia haemat 2 (1 1958
211 Olesen E B Olesen K Livingstone F B Cohen F Zuelzer W W Robinson A R and Neel J V Thala emia in Liberia Brit M J 1 1385 1959
12 Neel J V Genetic a pects of abnormal hemoglobins *Conf nce on Hemoglobin* p 53 National Academy of Sciences National Research Council Washington 1958
213 Ceppellini R *Proceedings VII Congress International Society of Hematology* (in press)
214 Singer K Josephson A M Singer L Heller P and Zimme man H Studies on abnormal hemoglobins XIII Hemoglobin S-thalassemia di ea and hemoglobin C-thala emia di ea e in siblings Blood 12 593 1 5
15 Itano H A Bergren W R and Stu geon P The abnormal human hemoglobins Medicin 35 1 1 1956
16 Aksoy M Abnormal haemoglobins in Turkey in *Ab o al Ha oglobin* p 16 Blackwell Oxford 1959
21 Romer M A and Dorken H D e neuen Erkennt sse ber d e geogr ph sche Verbreitung der Thalassamie (mit Untersuchung bei Lers rt und Turk n) Folia haemat 1 96 1956
218 Louya Y Thalassaemia in Iran in *Abnormal Ha n globi* p 73 Bl kw ll Oxford 1959
19 Matoth Y Shamir Z and Freundl h E L Thalass n n J ws from Kurdistan Blood 10 1 6 19 5
220 Chatterj a J B Haemoglobinopathy in India in *Ab o al Hae ogl* p 3 Bla kwell Oxford 1959
2 1 Minn ch V Na Nakorn S Ch ng h eon k S an l Kocha eni S Mediterran an anemia a stu ly of thirty two ca es in Thailand Blood 9 1 1 4
22 Chernoff A I The di tribution of the thalassemia gen a hi t r cal review Blood 14 899 1959

171 Lehmann H Distribution of the sickle cell gene A new light on the origin of the Last Africans Eugenics Rev 46 101 1954
172 Lehmann H and Raper A B The maintenance of different sickling rates in similar populations J Physiol 133 15P 1956
173 Edington G M and Laing W N Relationship between haemoglobins C + S + malaria in Ghana Brit M J 2 143 1957
174 Lehmann H Variations in human haemoglobin synthesis and factors governing their inheritance Brit M Bull 15 40 1959
175 Haldane J B S Disease and evolution Ricerca sci 19 Suppl 68 1949
176 Cooley T B and Lee P Series of cases of splenomegaly in children with *anemia and peculiar bone changes Tr Am Pediat Soc 37* 29 1925
177 Whipple G H and Bradford W L Mediterranean disease—thalassemia (erythroblastic anemia of Cooley) J Pediat 9 279 1936
178 Rietti F Les ictères hémolytiques avec augmentation de la résistance globulaire Ann méd 41 405 1937
179 Greppi E Ittero emolitico familiare con aumento della resistenze osmotica Minerva med 8 1 1928
180 Micheli F Penati F and Momigliano-Levi G Ulteriori richerche sull anemia ipocromica splenomegalica con poichilocitosi Atti soc ital hematologia in hematologica 16 5 1935
181 Angelini A Primi resultati di richerche ematologische nei familiari di ammalati di anemia di Cooley Minerva med 2 331 1937
182 Caminopetros J Récherches sur l anémie erythroblastique infantile des peuples de la Méditerranée Orientale Ann méd *43 27 104 1938*
183 Gatto I Richerche sui familiari di bambini affetti da malattia di Cooley Soc *ital ped (ez sicil) 14 12 1941*
184 Wintrobe M M *Clinical Hematology* p 517 Lea & Febiger Philadelphia 1942
185 Dameshek W Familial Mediterranean target-oval cell syndromes Am J M Sc 205 643 1943
186 Strauss M B Daland G A and Fox H J Familial microcytic anemia Observations on 6 cases of a blood disorder in an Italian family Am J M Sc 201 30 1941
187 Valentine W N and Neel J V Hematologic and genetic study of the transmissions of thalassemia (Cooley s anemia Mediterranean anemia) Arch Int Med 74 185 1944
188 Valentine W N and Neel J V A statistical study of the hematologic variables in subjects with thalassemia minor Am J M Sc *216 456 1948*
189 Silvestroni E Bianco I and Vallisneri E Nuovo contributo allo studio della questione genetica del morbo Cooley Minerva ped 1 136 1949
190 Silvestroni E and Bianco I Contributions to the modern knowledge of certain diseases of the blood and constitutional hematological abnormalities Sc med ital 2 269 1951
191 Rich A Studies on the hemoglobin of Cooley s anemia and Cooley s trait Proc Nat Acad Sc 38 187 1952
192 Bannerman R M Grinstein M and Moore C V Haemoglobin synthesis in thalassaemia Brit J Haemat 5 102 1959
193 Rimington C Some aspects of haemoglobin biosynthesis and their importance to medicine Brit M J 1 1017 1958
194 Stich W Die kongenitale Porphyrie Eine erythropathische hämolytische *Anamie—Porphyrocytose Schweiz med Wchnschr 88 1012 1958*
195 Schmid R Brecher G and Clemens T Familial hemolytic anemia with erythrocyte inclusion bodies and a defect in pigment metabolism Blood 14 991 1959
196 Frontali G and Stegagno G A Durée de la vie du globule rouge dans l anémie Mediterranean Helvet pediat acta 6 71 1951
197 Sturgeon P and Finch C A Erythrokinetics in Cooley s anemia Blood 12 64 1957
198 Bailey I S and Prankerd T A J Studies in thalassaemia Brit J Haemat 4 150 1958

## Chapter 35

# The Blood clotting Factors

*Rosemary Biggs and Lamont W Gaston*

This chapter is concerned with the clotting of the blood with the specific factors which normally participate in that complicated process and with those diseases which appear to have a genetic basis and which arise because of specific deficiencies in the clotting factors It must be said at once that the clotting factors are not known as specific chemical species (excepting $Ca^{++}$) and the reactions which they undergo or catalyze are known only phenomenologically Accordingly the diseases of the clotting of the blood cannot be given the precise biochemical definition that is the goal of science that level of understanding must await the painstaking work of the coming years The reviewers are perforce satisfied to provide a summary of the current status of a problem which is decidedly unfinished business

Flow of blood from a minor injury usually stops spontaneously and the shed blood clots at about the same time as the blood flow ceases Moreover if the blood flow fails to stop in many instances a solid clot also fails to form These commonplace observations suggest that the clot stops the flow Indeed the whole superstructure of blood coagulation theory is based upon the assumption that there is a causal relationship between hemostasis and coagulation It may come as a surprise that this fundamental tenet has been verified only experimentally through the study of hemorrhagic states This indirect approach to normal coagulation and hemostasis through study of the hemorrhagic diseases is made necessary by unusual experimental limitations The extent of these limitations must be understood if the problem of hemostasis is to be viewed in reasonable perspective

Normal blood in a glass tube will clot Formation of the clot may be observed at different temperatures in recalcified plasma in containers with different surfaces and in plasma with and without platelets If the sample of blood has defective coagulation the scope for study is immedi

223  Feng L I L Pathological haemoglobins in Indonesia in *Abnormal Haemoglobins* p 368 Blackwell Oxford 1959
224 Stransky E Foreign newsletter—Philippine Islands Blood 7 574 1952
225 Bianco I Montalenti G Silvestroni L and Siniscalo M Further data on genetics of microcythaemia or thalassaemia minor and Cooley s disease or thalassaemia major Ann Eugenics 16 299 1952
226 Sijpesteijn J A K Enige vormen van erfelijke niet spherocytaire anaemie bij kinderen Oppenheim Groningen 1958

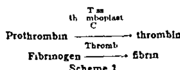
TABLE 35-1 COAGULATION FACTORS AND SYNONYMS

| *Most common name* | *Synonyms* | *Numerical term* |
|---|---|---|
| Fibrinogen | | Factor I |
| Prothrombin | | Factor II |
| Thromboplastin | Thrombokinase | Factor III |
| Calcium ion | $Ca^{++}$ | Factor IV |
| Factor V | Labile factor proaccelerin ac globulin | Factor V |
| Factor VI | Term no longer used | Factor VI |
| Factor VII | Stable factor proconvertin SPCA (serum prothrombin conversion accelerator) | Factor VII |
| Antihemophilic globulin | AHG antihemophilic factor (AHF) thromboplastinogen platelet cofactor I | Factor VIII |
| Christmas factor | Plasma thromboplastin component (PTC) | Factor IX |
| Stuart factor | Prower factor Stuart Prower factor | Factor X |
| Hageman factor | | |
| Plasma thromboplastin antecedent (PTA) | | |
| Thrombin | | |

## THEORY OF BLOOD COAGULATION

### CLASSICAL THEORY

The modern era of blood coagulation research began with the classical theory at the end of the last century According to this postulated scheme blood clotting follows two simple reactions




Scheme 1

The basis for this formulation was sound and much subsequent work supported it It is not proposed to discuss its foundation but to take it as an established starting point for modern developments The history of the theory of blood coagulation has recently been reviewed elsewhere [1]

According to this theory prothrombin is the one precursor of the coagulant thrombin The reaction is accelerated by a tissue extract called thromboplastin and requires the presence of $Ca^{++}$ The thromboplastin used experimentally is usually a saline extract of fresh or dried brain or lung tissue or on occasion the venom of the Russell s viper (RVV) The venom is a powerful coagulant which differs in its activity

ately increased Abnormal and normal samples may be mixed The normal sample may be fractionated to see which component will correct the abnormality in the abnormal sample When the fraction has been isolated and identified a reconstructed normal system can be devised in which the effects of omitting and including the pertinent fraction can be studied It is then usually possible to suggest a theory of *normal* coagulation to include the plasma fractions under study Invention at this stage may suggest a method for studying and even measuring the abnormality in other similar patients In this way most of the normal coagulation factors have been discovered and most useful techniques devised

As more and more different types of defect are studied the number of possible mixture experiments increases and the theory becomes more complex to accommodate all the newly postulated factors in a new *normal* scheme This evolution proceeds through phases of comparative clarity when most observed facts fit some hypothetical scheme and phases of revolutionary change when the hypotheses receive complete revision and the whole significance and interpretation of techniques must be reap proached Thus a test which was used with confidence as a measure of prothrombin at one phase may be interpreted as a quantitative measure of factor V or VII at a later time

The uncertainty of the whole approach has naturally not commended the subject to the biochemist and it must be admitted that the whole superstructure of theory and hypothetically interpreted methods has become very large In fact the inclusion of this subject in a book which deals mainly with the biochemical basis of inherited diseases may be unwarranted However the subject appears to be in an interesting phase which may be approaching transition a phase when a review emphasizing difficulties and inconsistencies may help to turn the kaleidoscope to a new and more intriguing pattern

It is proposed to begin the survey with a brief and undocumented account of the history of the theory of blood coagulation and to use it as a skeleton for reference during a more detailed consideration of the coagula tion factors and hemorrhagic states Details of technique will be intro duced as the need arises Finally an attempt will be made to relate the information reviewed to the role of coagulation in hemostasis A listing of coagulation factors and synonyms appears in Table 35 1

The subject matter will be limited to those inherited defects which are reasonably clearly defined For this reason reference to inhibitors of blood coagulation has been omitted patients with inherited abnormality of the inhibitors of coagulation are too rarely encountered to merit dis cussion The platelet defects classed as thrombopathy are omitted be cause the literature on this subject is still confusing and the different entities are not clearly defined

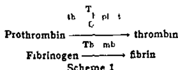
TABLE 35-1 COAGULATION FACTORS AND SYNONYMS

| Most common name | Synonyms | Numerical term |
|---|---|---|
| Fibrinogen | | Factor I |
| Prothrombin | | Factor II |
| Thromboplastin | Thrombokinase | Factor III |
| Calcium ion | $Ca^{++}$ | Factor IV |
| Factor V | Labile factor proaccelerin ac globulin | Factor V |
| Factor VI | Term no longer used | Factor VI |
| Factor VII | Stable factor proconvertin SPCA (serum prothrombin conversion accelerator) | Factor VII |
| Antihemophilic globulin | AHG antihemophilic factor (AHF) thromboplastinogen platelet cofactor I | Factor VIII |
| Christmas factor | Plasma thromboplastin component (PTC) | Factor IX |
| Stuart factor | Prower factor Stuart Prower factor | Factor X |
| Hageman factor | | |
| Plasma thromboplastin antecedent (PTA) | | |
| Thrombin | | |

## THEORY OF BLOOD COAGULATION

### CLASSICAL THEORY

The modern era of blood coagulation research began with the classical theory at the end of the last century According to this postulated scheme blood clotting follows two simple reactions



Scheme 1

The basis for this formulation was sound and much subsequent work supported it It is not proposed to discuss its foundation but to take it as an established starting point for modern developments The history of the theory of blood coagulation has recently been reviewed elsewhere [1]

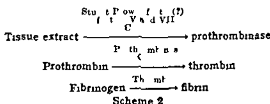
According to this theory prothrombin is the one precursor of the coagulant thrombin The reaction is accelerated by a tissue extract called thromboplastin and requires the presence of $Ca^{++}$ The thromboplastin used experimentally is usually a saline extract of fresh or dried brain or lung tissue or on occasion the venom of the Russell s viper (RVV) The venom is a powerful coagulant which differs in its activity

from mammalian tissue extracts (see below) Thrombin converts fibrinogen to fibrin which forms the matrix of the clot

## FIRST MODIFICATION FACTORS V AND VII AND THE STUART PROWER FACTOR

This convenient way of accounting for clotting was jolted by observations made over a period of several years that certain patients with faulty clot formation were defective in one of three specific factors necessary to the rapid conversion of prothrombin to thrombin These factors are now known as factors V and VII and the Stuart Prower factor In these cases examination of the blood showed a slow conversion of prothrombin to thrombin in the presence of tissue thromboplastic extracts and $Ca^{++}$ *Biggs Douglas and Macfarlane [2] and Hardisty [3] have shown that* factors V and VII in the presence of $Ca^{++}$ react with mammalian tissue extract thromboplastin to form an active substance which converts prothrombin to thrombin Owren, Rapaport Hjort and Aas [4] found that the product of this reaction can be sedimented by centrifuging as an *active particulate material*

Stuart Prower factor resembles factor VII in its physicochemical characteristics its deficiency on a hereditary basis gives rise to a hemorrhagic state indistinguishable clinically from factor VII deficiency The mechanism by which these three factors allow tissue thromboplastic extracts to effect the rapid conversion of prothrombin to thrombin is unclear Thus the classical theory had to be rewritten with the product of interaction of tissue extract $Ca^{++}$ factors V, VII and Stuart Prower factor being termed *prothrombinase*



Scheme 2

The term prothrombinase has been used by Owren [4] for the direct activator of prothrombin Russell s viper venom is not entirely interchangeable with tissue extract in forming prothrombinase The plasma from patients with factor VII deficiency (see below) reacts normally *if* PVV lipid [1] and $Ca^{++}$ are used brain extract fails to activate the clotting system fully It seems probable that RVV requires lipid and both factor V and the Stuart Prower factor for optimum activity Experiments *with*

[1] Lipid is any one of several fatty substances such as cephalin

RVV probably have no physiologic significance but it can be employed for diagnostic purposes with various pathologic samples (Table 35 2)

TABLE 35-2 PROPERTIES OF FACTORS V AND VII AND THE STUART PROWER FACTOR

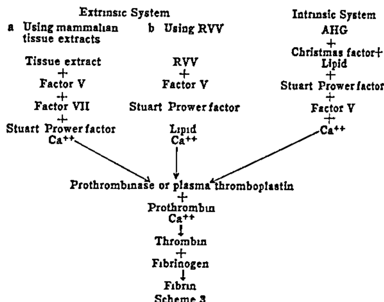
| *Properties* | *Factor V* | *Factor VII* | *Stuart Prower factor* |
|---|---|---|---|
| Stability on storage | Unstable | Stable | Stable |
| Presence in normal serum | Absent | Present | Present |
| Adsorption by inorganic salts | Not adsorbed | Adsorbed | Adsorbed |
| Required for normal action of | | | |
| 1 Brain extract | Required | Required | Required |
| RVV | Required | Not required | Required |
| 3 Plasma thromboplastin | Required | Not required | Required |

## SECOND MODIFICATION HEMOPHILIA AND CHRISTMAS DISEASE FACTORS AND BLOOD THROMBOPLASTIN FORMATION

An unsatisfactory feature of the classical theory even in its modified form was that it paid no attention to hemophilia the most important single coagulation defect In hemophilic blood all the factors envisaged by the classical theory are present in normal amounts In order to study the hemophilic defect it was necessary to ignore the theory and to study the subject from first principles

An early observation was that a fraction of normal plasma containing fibrinogen (Cohn fraction I) would correct the hemophilic abnormality in vitro The correcting substance came to be called antihemophilic globulin (AHG) It would not work properly without platelets Later it was found that a very powerful activator of prothrombin resembling prothrombinase could be formed from reagents derived entirely from normal blood This substance is called plasma thromboplastin At first it was thought that the substances necessary for plasma thromboplastin formation were AHG platelets factor VII factor V and $Ca^{++}$ but later work showed that this view was incorrect Factor VII is not required for plasma thromboplastin formation but two factors the Christmas factor (plasma thromboplastin component or PTC or factor IX) and the Stuart Prower factor are required

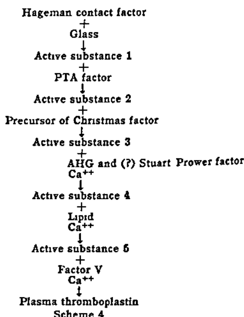
It is evident that the tissue and plasma prothrombin converting systems are similar and have certain components in common but they are different in their origin and in some of their requirements For convenience the system involving tissue extracts or RVV is called the extrinsic thromboplastin system and that derived wholly from blood constituents is called the intrinsic thromboplastin system The scheme can now be visualized as follows



Scheme 3

### THIRD MODIFICATION SURFACE CONTACT, PLASMA THROMBOPLASTIN ANTECEDENT (PTA) AND THE HAGEMAN FACTOR

If whole normal blood is collected into glass and silicone treated tubes the blood in the silicone tube takes longer to clot than that in the glass



Scheme 4

tube Some reaction occurs on exposure of blood to glass Evidence now suggests that two factor the Hageman factor and the PTA factor are concerned in the changes brought about by glass Probably the Hageman factor is activated by contact The activated substance then reacts with the PTA factor to produce another active product This product is probably concerned in the activation of the Christmas factor

To conclude this summary a sequence of possible reactions is given above It should be realized that this scheme is highly hypothetical The five active intermediate products proposed may have no basis in reality and indeed the whole process may be found to involve a series of parallel reactions rather than a sequential chain of reactions

## CLINICAL COAGULATION DEFECTS

*Afibrinogenemia*

A brief glance at any of the coagulation schemes will show that there can be no clot when there is complete absence of fibrinogen from the blood This particular defect is extremely rare

The diagnosis of afibrinogenemia is easily made clinically Plasma from such a patient does not clot upon the addition of thrombin nor is there a visible precipitate with 25 per cent $(NH_4)_2SO_4$ fractionation

Typical cases have been described by Macfarlane [5] and Fernando and Dharmasena [6] Neither of these patients was a severe bleeder Both bled at the separation of the umbilical cord and from superficial abrasions Neither had any hemarthroses or deep muscle hematomas One (the patient of Fernando and Dharmasena) was admitted to hospital and operated on successfully for hemoperitoneum The other died at the age of 28 from retroperitoneal bleeding

Of great interest has been the mildness of the hemorrhagic diathesis in some of the reported cases Some of the females have had normal menses and the unusual complication of hemarthrosis does not seem to result in permanent crippling of the joint

The generation of thromboplastin and thrombin has been found to be normal it seems that only the synthesis of fibrinogen is defective Gitlin and Borges [7] have shown that the half life of fibrinogen infused into two such patients is approximately 4 days a survival comparable to that in normal individuals

Genetics The two patients mentioned and many of the others recorded were the offspring of consanguineous marriages Both sexes are affected the defect being transmitted as an autosomal recessive trait Patients exhibiting the clinical picture are considered to be homozygous for the defect Multiple cases within a family have been reported [8] Low levels of fibrinogen have been found in those presumed to be heterozygous

carriers of the defect but the evidence that heterozygous carriers can thus be detected has been questioned [9]

*Prothrombin Deficiency*

Prothrombin deficiency as a single defect is so rare as to be worth mention only Biggs and Douglas [10] recorded a case of acquired deficiency in a man and Quick Pisciotta and Hussey [11] have described three patients whose defect was probably prothrombin deficiency Two of the patients were brothers who had normal parents four normal siblings and normal maternal and paternal grandparents One bled for 2 months following circumcision bruised easily bled after tooth extractions and had a probable hemarthrosis as well as hematuria The second had suffered from posttraumatic bleeding gastrointestinal bleeding easy bruising and two episodes of bleeding into his calf muscles The latter incident responded clinically to injections of vitamin K Both boys had prolonged prothrombin times which in the case of the second could be shortened by administration of vitamin K Both boys had normal levels of factor V and VII No two-stage prothrombin time test was done to confirm the prothrombin deficiency The third patient of Quick and coworkers was a 5-year old girl of an unrelated and entirely normal family Mixing her plasma with that of the first boy gave no shortening of the prothrombin time

DEFECTS IN THE EXTRINSIC THROMBOPLASTIN SYSTEM

The study of the deficiency states of the extrinsic thromboplastin system began in one of the periods of relative clarity and simplicity of blood coagulation theory In 1935 Quick [12] devised the one stage prothrombin time test This test was based on the classical theory of blood coagulation (Scheme 1) It consists of adding a potent tissue extract to plasma and thereafter recalcifying Whereas the clotting time of normal plasma in the presence of $Ca^{++}$ alone may be 120 to 180 sec the addition of tissue extract reduces the clotting time to 12 to 15 seconds According to the classical theory this shortening of the clotting time is due to the accelerated conversion of prothrombin to thrombin If the clotting time is reduced in this way to a minimum and if fibrinogen is present in adequate amounts it follows from the classical theory that variations in clotting time are caused by variation in prothrombin content Thus the one stage prothrombin time was used as a measure of prothrombin concentration

It is now recognized that the one stage prothrombin time test practically never gives a measure of prothrombin itself Prothrombin in normal blood is absent from serum because it is used up during clotting It is also adsorbed from plasma by inorganic salts such as $BaSO_4$ $BaCO_3$ and $Al(OH)_3$ If to a plasma sample with a long one stage prothrombin

time is added either serum or adsorbed plasma there should be no shortening of the one stage prothrombin time providing the defect is pure prothrombin deficiency Actually the clotting of practically all pathologic samples is accelerated by addition of normal serum or adsorbed plasma Thus the defect in these patients is not of prothrombin itself but of some other factor in the thromboplastin system

In addition prothrombin can be measured by a method which is independent of the factors other than prothrombin such as factor VII Stuart Prower factor factor V etc This is the two stage prothrombin time test Its use also indicates that pure prothrombin deficiency is very rare It is performed by adding tissue thromboplastin to platelet poor plasma and recalcifying As thrombin is formed aliquots are taken at serial times for addition to tubes containing standard amounts of fibrinogen From a previously constructed thrombin fibrinogen calibration curve the amount of thrombin added can be deduced and the prothrombin in the initial sample calculated

*Factor V Deficiency*

In 1947 Owren [13] described a female patient whose plasma had a long one-stage prothrombin time The clotting time was reduced practically to normal by adding adsorbed (prothrombin poor) plasma Owren showed that the corrective factor was in the fraction of adsorbed plasma which precipitated between 33 to 50 per cent saturation with $(NH_4)_2SO_4$ Since it differed from all previously described factors he called it factor V The factor is very unstable on storage in the presence of oxalate It is not adsorbed by inorganic precipitates It disappears during clotting and is therefore not present in serum The plasma of a patient with factor V deficiency has a long one stage prothrombin time if RVV is used as thromboplastin

Clinical Findings Patients with this defect may be severe bleeders Nose bleeding and massive bruises are common After trauma the bleeding may be delayed for several hours but thereafter persists until healing is advanced Scratches and small puncture wounds do not bleed excessively Menorrhagia is a cause of serious anemia in females Hemarthroses are uncommon Other cases of factor V deficiency are referred to by Biggs and Macfarlane [1]

Genetics The disease may occur in either sex Sometimes more than one member of the family is affected Relatives of affected members may show subclinical deficiencies of factor V In other instances no other members of the family show any abnormality The disease may occur in either sex and may be mild or severe in degree Although sporadic cases occur a familial pattern is more common Consanguinity has been noted in some instances but not in others Persons with mild cases of factor V deficiency have been found to have 1 to 5 per cent of normal levels of

factor V, severely affected patients have demonstrated no detectable levels Congenital deficiency of factor V has been reported in association with congenital heart disease [14] syndactylism [15] epidermolysis bullosa congenitalis [16] and decreased levels of prothrombin [15] and AHG [17] There is as yet no general agreement on the mechanism of transmission An excellent discussion of the inheritance of factor V deficiency and of other inherited clotting disorders is given by Ratnoff [18]

*Factor VII Deficiency*

Only a few patients with a congenital bleeding tendency attributable to factor VII deficiency have been described The defect is suspected if there is a long one-stage prothrombin time using brain extract as thromboplastin and if the clotting time by this test is reduced to near normal by the addition of normal serum The uncomplicated nature of the defect is confirmed in modern terms if a normal blood thromboplastin forming system is demonstrated and if the one-stage prothrombin time using RVV as thromboplastin is also normal

Factor VII is stable on storage and is adsorbed from serum by inorganic precipitates On electrophoresis factor VII activity is found in the β globulin fraction

**Clinical Findings** The cases of Hicks [19] Ackroyd [20] and Pitney [21] are well described Ackroyd s patient a male had severe epistaxis and two hemarthroses He bled from small cuts in a rather peculiar way The lesions would stop bleeding normally but bleeding would restart in 3 to 4 hr In later life he developed gastrointestinal bleeding An operation on an ankle caused no undue bleeding Hicks patient a female bled at the time of eruption of her first teeth She had epistaxes in childhood and later menorrhagia Three major operations two for umbilical hernia and one for appendicitis were performed without undue bleeding Pitney s patient also a female bled sufficiently to require transfusion after dental extractions She bled excessively after eight deliveries Dental extraction following plasma transfusion therapy was uneventful

**Genetics** The patients described above had no history of bleeding in other members of the family

Deficiency of factor VII has been noted both on a sporadic and on a ✓ familial basis In a family reported by Ratnoff the deficiency appeared to be inherited as a recessive trait and the heterozygous carrier could be detected A similar situation was reported by Quick Pisciotta and Hussey [11] who described a 3 year-old girl who had been a severe bleeder since birth She was found to be factor VII-deficient Her brother parents and maternal grandparents were clinically normal yet all but the maternal grandmother had mildly prolonged prothrombin times and were assumed to be heterozygous for the defect Careful studies of factor VII-deficient families by Voss [22] and Dische [22a] also suggest the factor

VII mutant gene is inherited as an autosomal recessive or intermediate gene Thus although the disease is congenital there is no general agreement about inheritance

*Stuart Prower Defect*

This abnormality has been defined in recent years from the work of Bergsagel [23] Telfer Denson and Wright [24] Hougie Barrow and Graham [25] Graham Barrow and Hougie [26] Bachman Duckert Geiger Baer and Koller [27] and Denson [28] There appear to be no specific distinguishing clinical features corresponding to this defect The abnormality is associated with a long one stage prothrombin time when either brain extract or RVV is used as thromboplastin The patient s blood also shows an abnormality in intrinsic thromboplastin formation (see below)

TABLE 35-3 DIAGNOSTIC TESTS FOR AFIBRINOGENEMIA AND FOR DEFICIENCIES OF PROTHROMBIN FACTOR V FACTOR VII AND THE STUART PROWER FACTOR

| Deficiency | Clotting time | Two stage prothrombin test | Prothrombin time | | Correcting effect of | |
|---|---|---|---|---|---|---|
| | | | Brain | RVV | Adsorbed plasma | Serum |
| Afibrinogenemia | No clot | Normal | No clot | No clot | Normal clot | No clot |
| Prothrombin deficiency | Long | Abnormal | May be normal | May be normal | No clot | No clot |
| Factor V deficiency | Long | Normal | Long | Long | Normal clot | No effect |
| Factor VII deficiency | Normal | Normal | Long | Normal | No effect | Normal clot |
| Stuart Prower deficiency | Long | Normal | Long | Long | No effect | Normal clot |

Genetics In the case of Hougie Barrow and Graham [25] the inheritance appeared to be through an autosomal recessive gene In this family the members heterozygous for the defect were found to have subnormal levels of Stuart Prower factor This was true also of the family described by Bachman et al [27] A pedigree for Stuart Prower factor deficiency has also been recently described by Roos Arkel Verloop and Jordan [29] This family included a consanguineous marriage which produced 12 children 6 of whom were bleeders These 6 had levels of Stuart Prower factor between 5 to 6 per cent The parents 4 of the 6 asymptomatic children and several asymptomatic relatives were found to have subnormal levels of Stuart Prower factor and were assumed to be heterozygous One female in this family was heterozygous for both Stuart Prower defect and alcaptonuria Thu of the three families all who were

heterozygous were asymptomatic or nearly so and those homozygous for the defect were bleeders

The properties of factors V, VII and the Stuart Prower factor related to the extrinsic thromboplastin system are compared in Table 35 2 the diagnostic tests used to distinguish between the anomalies are given in Table 35 3

## DEFECTS OF THE INTRINSIC THROMBOPLASTIN SYSTEM

### *Hemophilia (Hemophilia A Antihemophilic Globulin Deficiency Factor VIII Deficiency)*

Because it is the best known and most common of the clotting defects hemophilia must occupy a central point of interest in any study of inherited disorders of blood coagulation The primary defect in this disease remains unsure The contemporary interpretation is that the blood of hemophilic patients lacks AHG a substance necessary for normal coagulation A minority view is that the defect is at least in part attributable to an inhibitory substance which depresses coagulation

Most of the evidence supporting the theory that an inhibitor is responsible for the hemophilic defect is indirect Thus the clotting of diluted and undiluted plasma is compared or comparisons are made of hemophilic and normal plasma as substrates in the thromboplastin generation test [30 31] These observations do not directly delineate an inhibitor An inhibitor in hemophilic plasma should destroy or mask AHG It is true that AHG disappears rapidly from the blood of treated hemophilic patients Various estimates assess the half life of AHG after treatment at 9 to 11 hr [12 33] Thus it is possible that hemophilic blood does contain an inhibitor but until the half life of AHG in normal blood is known there can be no certainty about this At present it has certainly proved of practical value to treat hemophilic patients as if the main defect is AHG deficiency

Probably more is known of the nature of AHG than of any of the other clotting factors except $Ca^{++}$ The substance lacking from hemophilic blood is very unstable Normal human blood collected for transfusion may lose much of its activity in 6 hr and the activity may be reduced to 30 per cent of the original in 24 hr The activity is better preserved if plasma is separated soon after collection and frozen at −20 C but even at this temperature some activity is lost Activity is usually also lost during freeze drying During coagulation AHG disappears This utilization or destruction during clotting may be in part responsible for the loss of activity during the ordinary collection of blood for transfusion because in collection a minimum amount of coagulation is usual and this may be sufficient to dispose of much AHG activity Careful attention to mixing during collection seems to reduce losses [34]

Fractionation by several different methods shows that AHG precipitates with the fibrinogen fraction of plasma No separation of activity from human fibrinogen has been achieved without gross loss of activity On the other hand the blood of patients with afibrinogenemia contains AHG [36] Electrophoresis of plasma from such a patient has shown that AHG separates with the $\beta$ globulin fraction [35]

AHG separated with fibrinogen has a narrow pH range of relative stability It is destroyed by heating and by storage at room temperature or at 0 C AHG is not adsorbed by inorganic precipitates such as $BaSO_4$ and $Ca_3(PO_4)_2$

Clinical Features The majority of hemophilic patients are males The disease in its classical form usually declares itself in early childhood when mobility exposes the child to increasing minor trauma Bruises disproportionate to the injury appear and disabling joint swellings may follow The painful joint confines the patient to bed and the muscles waste During convalescence the joints may be poorly protected and hemarthroses commonly recur A self perpetuating cycle of hemarthroses and wasting is initiated until by adolescence the young man may have already acquired deformed and weakened limbs Equally disabling and often more dangerous bleeding into muscles particularly the retroperitoneal muscles may occur at any time Hematuria gastrointestinal bleeding and epistaxis are common Trauma such as tongue biting small cuts and dental extractions all give rise to troublesome and prolonged bleeding which may require transfusion

A characteristic feature of the bleeding in hemophilic patients is that it occurs in phases The patient in a good phase may live almost normally for months and sometimes years surmounting many minor injuries with little inconvenience At other times massive and apparently spontaneous bruises appear The least injury may produce hemarthroses and further lesions appear while the patient is resting in bed to recover from the first No consistent changes in the hemostatic mechanism have been found to account for these variations

Another feature of hemophilic bleeding is its delayed onset If an operation is carried out on an untreated hemophilic patient the surgeon is often pleasantly surprised by the freedom from bleeding at the time of operation This happy state is often interrupted 6 to 8 hr later by a steady slow and persistent flow of blood The surgeon returns to search for bleeding points and usually some bleeding vessel is found and ligated Such operations are often unsuccessful and a continuing battle against a leaking sieve may continue with constant blood replacement until the much delayed healing finally happens or death occurs Even the treated patient is susceptible to secondary hemorrhage usually from apparently irrelevant small vessels A single tooth socket may bleed for 4 to 6 weeks if no specific treatment is given

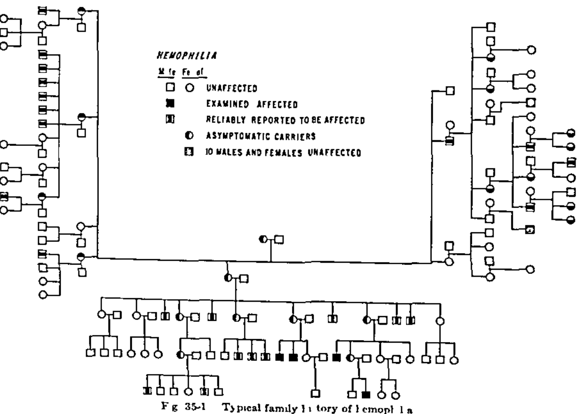



Fig 35-1 Typical family history of hemophilia

This i the general clinical picture of bleeding in the severely affected patient but many hemophilic patients have a much milder version of the disea e In ome the defect is disclosed only by serious injury, when unexpected bleeding stimulates a laboratory study

Genetics Hemophilia is inherited as a sex linked recessive charac teristic A typical family record is shown in Fig 35 1 The vast majority of families with more than one affected member show a pattern con istent with this interpretation Exceptional families in which an occa sional female has a mild bleeding tendency are often interpreted quite satisfactorily on the hypothesis that the gene is incompletely rece ive

Several ca es of apparently true hemophilia in females have been reported Three si ters who were the off pring of a consanguineous marriage in a hemophilic family were reported by Merskey [36] They suf fered from post wound and post tooth extraction bleeding hematuria and ga trointestinal bleeding but survived childbirth and bore hemophilic sons The female reported by I raels [37] was born of unrelated parents who were a hemophiliac and a hemophilia carrier re pectively A recent case of sporadic AHG deficiency in a 6 year-old white female has been reported by Quick and Hus ey [38] A colony of hemophilic dogs which has been maintained at the University of North Carolina for several years has produced hemophilic females following the crossing of a hemophilic male and a carrier female [39] Families studied often how an apparent excess of affected males [40] but this disproportion is probably due to bias in selection No family is studied unless at least one affected male has been discovered and thus families of carriers fortunate enough to have all normal children are omitted

Nearly half the families studied have only one affected male There are two interpretations for this phenomenon Either the members of the family are ignorant about affected ancestors and distant cou ins or the disea e has arisen anew by mutation There are very few records suffi ciently complete for certain distinction between the e possibilities Cal culations based on the life expectation of hemophilic males which is variously estimated at 16 to 22 years indicate that mutation mu t be fairly frequent because there is little obvious tendency for the di ea e to decrea e

*Christmas Disease* (Pl a ma Thromboplastin Component or PTC Defi ciency Hemophilia B Factor IX Deficiency)

This condition re embles hemophilia o closely that until recently the two abnormalities were not eparated The clinical manife tations of the two are identical as is the mode of inheritance The conditions are distingui hed becau e a mixture of equal parts of blood from patients with hemophilia and Chri tmas di ea e clots normally wherea the eparate amples clot abnormally The two defects are caused by defi

*ciency of different factors [41, 42] Little is known of the nature of the* factors

**Clinical Features** Little needs to be said about the clinical features of Christmas disease since they are identical with those of hemophilia Among the senior author's patients there are more mild cases of Christmas disease than of hemophilia This may be a local phenomenon A large proportion of the mildly affected patients are derived from one area Although there is no evidence for relationship between the different families, it seems very probable that there may be common ancestors It should not be thought on this account that Christmas disease is a milder defect than hemophilia Some Christmas disease patients are very severely affected

A major and unique contribution to the study of Christmas disease comes from Switzerland [43 44] The geographic social and religious organization of residents in the Tenna Valley is such that relationship of a family can be traced back with considerable certainty for 11 generations In this family 3 072 members, including 55 bleeders have been recorded Moor-Jankowski [43, 44] and his colleagues have brought the records of this family up to date and have personally studied 10 bleeders All these patients had Christmas disease and Christmas factor levels of only 2 5 to 6 per cent of normal Thus from the laboratory point of view there is great uniformity in the expression of the disease Nonetheless there were marked differences among the different bleeders Some were severely affected from early childhood others had no symptoms for many years The authors also noted a general tendency for the bleeding to be less severe with advancing years The average life expectancy of affected members is 22 years

**Genetics** The inheritance of Christmas disease is the same as that of hemophilia as a sex linked recessive The study of the Tenna Valley family in Switzerland adds much to detailed understanding [43 44] This family has one case of Christmas disease that must be regarded as arising through mutation A strange finding in this family is that female carriers have significantly more children than the normal females In this family there is no natural tendency of the disease to die out even without mutation The decreased number of offspring of affected males is almost exactly balanced by the increased fertility of the carriers This record is invaluable because it provides a really good basis for testing hypotheses about inheritance in an exceptionally well recorded and studied single family

*Surface Contact* (Plasma Thromboplastin Antecedent (PTA) Deficiency and the Hageman Defect)

The effect on clotting of contact with glass is well recognized This phenomenon suggests that glass brings about some alteration in the

blood Recent studies have connected this phenomenon with two path ologic blood conditions PTA deficiency and the Hageman defect [45–51] The e defects are currently considered together

**PTA Deficiency** In 1953 Rosenthal Dreskin and Rosenthal [46] described a new hemorrhagic state which appears to be a re ult of PTA deficiency and is inherited as an autosomal dominant characteristic All four patients with this syndrome that have been seen in the senior author s clinic have been Jewish This observation may have no signifi cance but if there is a true association with Jewish ancestry the variable distribution of the disease in different parts of the world would be ex plicable The disease is for example common in New York but uncom mon in England [40]

Patients with PTA deficiency are mostly rather mild bleeders In two female patients of the authors menorrhagia necessitated hysterectomy All have had relatively marked bruising tendency and all have bled after dental extractions or operations Occasionally more severely affected patients have had hemarthroses or muscle hematomas

**The Hageman Defect** [47] This syndrome is a strange and subversive phenomenon in the blood clotting field The eponym derives from the name of the first patient studied Patients with no hemostatic defect clinically have occasionally been found with gross laboratory abnormali ties The absence of clinical defect has been confirmed in all cases by multiple uneventful surgical operations The condition therefore is readily distinguished from PTA deficiency in which there is bleeding The abnormality appears to be familial but the mode of inheritance is unclear

**Physiologic Interpretations** It is convenient to consider these two abnormalities together because the laboratory results are similar Blood samples from patients with either syndrome may have a long clotting time and an abnormal prothrombin consumption In both abnormalities the thromboplastin generation test shows an unusual pattern but the re ults are often abnormal only if both serum and adsorbed plasma are derived from the patient In some cases a slight apparent deficiency of Christmas factor occurs In others the thromboplastin generation test gives entirely normal results

Conflicting results have emerged from many aspects of laboratory testing in these cases Fractionation work has not revealed a specific PTA fraction with any clear definition Samples initially apparently deficient in PTA factor may approach normality on storage Mixture experiments may give confusing results samples from the same patient may be considered to show PTA deficiency or Hageman defect on different occasions Many of these difficulties can be attributed to the nature of the underlying defect

In both these conditions there appears to be a defect in ability to react

to glass surface Thus depending on the degree of glass exposure of samples being tested the results will vary From the observations of Biggs et al [45] it seems that if samples are collected in silicone treated apparatus a glass exposed PTA deficient sample will correct the defect of a silicone-stored sample from a Hageman defect patient but will not correct the abnormality of a silicone-collected PTA deficient specimen In the reverse experiment a glass-exposed sample from a Hageman defect patient will not correct the abnormality of a silicone-collected PTA deficient specimen These experiments suggest that the Hageman factor is the one which reacts to contact with glass and that the product of this activation reacts with the PTA factor

The apparent Christmas factor deficiency which may be encountered in these cases is probably due to slow activation of a precursor of the Christmas factor If a mixture of PTA deficient and Christmas disease serums is allowed to stand before testing the abnormality may disappear [45] It presumably takes some time for the PTA factor in the Christmas disease serum to activate the precursor of Christmas factor in the PTA deficient serum

## MULTIPLE FACTOR DEFECTS

### *von Willebrand s Disease*

This disease would not previously have been included among the constitutional coagulation defects because the main abnormality has always been considered a failure of capillary function It is now recognized that many patients with this syndrome are also deficient in AHG A new name for this disease vascular hemophilia has recently gained favor but since the original family described by von Willebrand [52] had AHG deficiency there would seem to be little ground for multiplication of nomenclature

In the more severely affected subjects there is evidence for a dual defect one of the clotting mechanism and the other of the capillaries Severely affected patients often have a level of AHG which is in the range seen in the blood in mild hemophilia but this association of defects is by no means found in all cases Nothing is known of the intimate nature of the capillary defect One possible explanation is that abnormality of the small vessels produces AHG deficiency when the vessel anomaly is sufficiently severe Perhaps such vessel lead to rapid utilization of AHG, or perhaps AHG is made in the normal vascular endothelium

**Clinical Features** Patients with this condition bruise easily They have frequent and often severe epistaxes Females may have severe menorrhagia which may require transfusion There may be bleeding from the gums and there is bleeding always after dental extraction Small cuts bleed freely but the blood flow is stopped easily and usually permanently by pressure Surgery has proved surprisingly uneventful There is

usually some excessive bleeding but the persistent and uncontrollable bleeding of the hemophilic patient is absent

Genetics The condition is inherited as an autosomal dominant trait A typical family tree is given in Fig 35 2 In mildly affected patients the level of AHG may be normal Nilsson Blomback and Francken [53] record a family in which a low AHG level occurred in association with von Willebrand s disease Study of clinically unaffected members of the

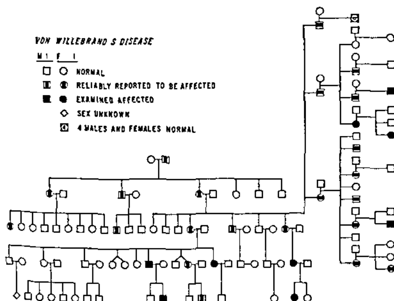



Fig 35 2 Family history illustrating the inheritance in von Willebrand s disease

family showed that some of them had low AHG levels There is thus the possibility that the two features (AHG level and capillary function) are inherited separately and that the combination gives rise to severe examples of disease Such severely affected patients are more frequently studied than less serious bleeders This possibility was suggested by Pitney [54] If this is the case this variety of AHG deficiency is inherited as an autosomal dominant characteristic

## *Dicoumarol Intoxication*

A most informative and relevant abnormality (although not an inherited disorder) is that caused by the dicoumarol group of drugs The type of clotting anomaly depends upon the duration of treatment and to some extent on the drug used A patient who has been treated for more than a week and whose level of prothrombin time is within the therapeutic range usually shows a pattern as follows

| *Factor* | *Percentage of Normal* |
|---|---|
| Prothrombin | 40-70 |
| Factor VII | 5-10 |
| Christmas factor | 10-50 |
| Stuart Prower factor | 10-50 |

In addition there may be deficiency of yet another coagulation factor, which is at present ill defined The level of Christmas factor and Stuart Prower factor depends upon the extent of the defect Exactly the same pattern is found in vitamin K deficiency and in some congenital cases with multiple clotting defects [55 56] These observations suggest that there must be some common pathway for the manufacture of these factors or that the defect represents an interference with some factor on which all the others depend The blood of these patients may show confusing results with conventional tests The clotting time is usually normal because none of the factors is reduced to a rate-limiting degree *Thromboplastin generation is often quite abnormal* when the *patient s serum* replaces normal serum Mixture experiments may give confusing results unless specifically defective samples are used

Other reported anomalies include combined deficiency of AHG and *factor V [17 58] or factor VII and Stuart Prower factor [28]*

## LABORATORY DIAGNOSIS OF CLOTTING FACTOR DEFECTS

*The clinical tests of clotting for the most part relate to measurements* of the components of the internal thromboplastin system Some of these will be reviewed here in order to indicate the extent of the current experimental limitations in the coagulation field

### *Clotting Time*

In this test a measured quantity of blood is put into a glass tube and the time until solidification is complete is recorded The details of technique vary from center to center Usually four tests are done and average results reported The test is entirely nonspecific any defect at any stage may influence the results The test has been modified for quantitative measurement of a specific factor by taking the blood of a patient with a known defect e g hemophilia and adding to this the blood of another patient If the clotting time of the mixture is shorter than that of the standard patient then the undiagnosed patient has some other defect By adding different known proportions some quantitative estimate can be made The use of the test in this way is open to much criticism and in practice is most inaccurate The test will for example *record normal* results with as little as 1 to 5 per cent of AHG in a sample of hemophilic blood Moreover the practice rests on the assumption that the defect in the standard plasma is accurately known This is certainly not often

true For example before the recognition of Christmas disease the random selection of a hemophilic plasma as a standard would not infrequently have resulted in the choice of a sample from a patient with Christmas disease

*Prothrombin Consumption*

During normal coagulation prothrombin is converted to thrombin and little remains in the serum If the intrinsic plasma thromboplastin forming system is defective then much prothrombin is found in serum The test consists in making an empirical assessment of the relative amount of prothrombin present in the serum The test is much more sensitive than the clotting time to minor deviations from normal The test is in itself quite nonspecific It gives no information about what is wrong but merely records abnormality It has been used with standard blood samples to define new defects in the manner described for the clotting time test

*Thromboplastin Generation*

In this test reagents prepared from normal plasma when mixed together can be shown to generate a powerful thromboplastic agent in the presence of calcium ions [62] The reagents required are $Al(OH)_3$ or $BaSO_4$ treated plasma serum and platelets To analyze the reactions involved it is necessary to consider the factors contained in this complicated mixture

**Al(OH) or BaSO treated Plasma** This contains AHG and factor V Experiments in which one or the other of these factors is excluded from the system have shown that both are essential

**Serum** Serum contains factor VII Christmas factor and the Stuart Prower factor Experiments this time with serum from patients who lack one or the other of these factors have shown that Christmas factor and the Stuart Prower factor are required but that factor VII is not essential

**Platelets** Platelets can be replaced in the thromboplastin generation test by preparations of brain phospholipids made according to the method of Folch [63] With this information there have been many attempts to identify a chemically definable fraction as the one active substance Some workers have found that attempts at purification are accompanied by loss of activity and have concluded that a single active substance cannot be located [64] Others have implicated phosphatidylethanolamine [65 66] and phosphatidyl serine [66–68] as the active compound Recent work suggests that several phosphatides may be required [68] a suggestion which would account for the loss of over all activity during purification

The thromboplastin generation test is used as a diagnostic aid In diagnosis it is used to distinguish between hemophilia and Christmas disease by making mixture reagents from normal blood and a patient s blood The general plan of such a diagnostic test is shown in Table 35-4 This test does not require a standard abnormal sample to define the

abnormality but it does rest on the assumption (probably false) that all the main coagulation factors are known

TABLE 35 4 THROMBOPLASTIN GENERATION TEST USED FOR DIAGNOSIS OF HEMOPHILIA AND CHRISTMAS DISEASE IN PATIENTS WHOSE PLASMA HAS A NORMAL ONE STAGE PROTHROMBIN TIME

| *Source of $Al(OH)_3$ treated plasma* | *Source of serum* | *Source of platelets or phospholipid* | *Results* | |
|---|---|---|---|---|
| | | | *Hemophilia* | *Christmas disease* |
| Normal | Normal | Normal | Control test | |
| Normal | Patient | Normal | Normal | Abnormal |
| Patient | Normal | Normal | Abnormal | Normal |
| Patient | Patient | Normal | Abnormal | Abnormal |

Both factor V and Stuart Prower factors are required for blood thromboplastin formation deficiencies of these two factors are distinguished by the thromboplastin generation test as shown in Table 35 5 In tests using these samples phospholipid [*69*] should be used in place of platelets because platelets adsorb factor V from normal plasma and the use of normal platelets may mask the defect

TABLE 35 5 THROMBOPLASTIN GENERATION TEST RESULTS FOR PATIENTS WITH FACTOR V OR STUART-PROWER DEFECT

| *Source of $Al(OH)_3$ treated plasma* | *Source of serum* | *Results* | |
|---|---|---|---|
| | | *Factor V deficiency* | *Stuart Prower defect* |
| Normal | Normal | Control test | |
| Normal | Patient | Normal | Abnormal |
| Patient | Normal | Abnormal | Normal |
| Patient | Patient | Abnormal | Abnormal |

The one-stage prothrombin time test carried out on the plasma of these patients gives abnormal results

Factor VII deficiency causes no blood thromboplastin defect thus clotting time prothrombin consumption index and thromboplastin generation test all give normal results

The thromboplastin generation test can also be adapted to study the physiology of blood thromboplastin formation Thus Biggs Douglas and Macfarlane [*70*] showed that if AHG serum and calcium are in

cubated together an active intermediate product of coagulation is formed The interaction of this product with platelets produces an active particulate substance which can be sedimented by centrifuging [71] Biggs and Bidwell [72] showed that the final product of thromboplastin formation is unstable but can be reactivated by the addition of factor V Hougie and his coworkers [25] have shown that the Stuart Prower factor is probably concerned together with Christmas factor in reaction with AHG

*Modification of the Thromboplastin Generation Test for the Assay of AHG* [32]

A method for the quantitative assay of AHG can be devised if it is assumed that AHG affects quantitatively the yield of active thromboplastin and that preparations which give the same minimum clotting time by this test have the same activity Using this method it has been possible to predict from blood levels of AHG the severity of bleeding to be expected Thus if a patient has more than 5 per cent of a normal level of AHG he will be very mildly affected Again if levels below 25 per cent of normal are achieved by therapy the bleeding will not be controlled adequately after surgical treatment From these practical results it is reasonable to assume that the test gives some measure of the hemophilic defect [32] It should be emphasized that a common sense assessment of success or failure in practice is the only way of judging whether or not assumptions about a quantitative method are reasonable

The election of techniques described is not comprehensive nor are they necessarily the best tests The main purpose of this review is to bring into focus the limitations of this whole field of study In Table 35 6 the methods have been related to the theory of blood coagulation It will be seen that even assays for specific factors may be affected by many variables and that the factors which affect the cruder screening tests such as the clotting time and prothrombin consumption tests, are innumerable In the more specific assays attempts are made to eliminate undesirable variables by using standard plasma samples or by supplying irrelevant factors at high concentration Despite this care most coagulation tests rank in accuracy and reliability more with rather crude biologic assays than with chemical analyses

## BLOOD COAGULATION AND HEMOSTASIS

There now remains the question of the relationship between hemostasis and blood coagulation The conventional view of hemostasis [73] is of a dual mechanism involving platelet capillary and arteriolar function on the one hand and blood coagulation on the other A small injury ceases to bleed because the vessels are contracted and plugged by plate

TABLE 35 6 APPROXIMATE RELATIONSHIP OF TESTS USED IN THE DIAGNOSIS OF COAGULATION DEFECTS TO THE STAGES OF BLOOD COAGULATION WHICH AFFECT THEM

| *Stages of the extrinsic thromboplastin system using mammalian tissue extract thromboplastin* | *Tests applicable to the extrinsic system* | *Tests applicable to both extrinsic and intrinsic systems* | *Tests applicable to the intrinsic system* | *Stages of the intrinsic thromboplastin system* |
| --- | --- | --- | --- | --- |
| | | Cl tt g t me test ↓<br>Proth o b n n mpt test ↓ | Glass act t t ↓<br>AHG assay ↓<br>Th mbo last n g n t n test ↓ | Hageman contact factor + glass<br>↓<br>Active substance 1 + PTA factor<br>↓<br>Active substance 2 + precur or of Christmas factor<br>↓<br>Active substance 3 + AHG and (?)Stuart Prower factor + $CaCl_2$<br>↓<br>Active substance 4 + lipid + $CaCl_2$<br>↓<br>Active substance 5 + factor V + $CaCl_2$<br>↓<br>Plasma thromboplastin + prothrombin + $CaCl_2$<br>↓<br>Thrombin + fibrinogen<br>↓<br>Fibrin |
| Brain extract + factor V + factor VII + Stuart Prower factor + $CaCl_2$<br>↓<br>Prothrombinase + prothrombin + $CaCl_2$<br>↓<br>Thrombin + fibrinogen<br>↓<br>Fibrin | O e t ge p tl mb t m ↓<br>T o-st g pr tl l t t ↓<br>T t f f t a V VII d S t tl f ct ↓ | Tl ml t n f tl m ↓ | | |

lets After a lapse of time the vessels relax and bleeding is not resumed because the vascular lumen is filled by clot For larger injuries vascular contraction cannot stem the flow and ligature or pressure must arrest the bleeding and allow time for blood coagulation There is no reason to suppose that this view is not substantially correct A detailed analysis of bleeding associated with single-factor defects may suggest whether or not hemostasis may be achieved by one of the several mechanisms available and whether or not the mechanisms are different in different tissues A study of some inconsistencies may also reveal new fields for exploration

By comparing factor VII deficiency with hemophilia or Christmas disease the relative importance of the intrinsic and extrinsic thromboplastin systems may be assessed The first point of interest is that two of the factor VII-deficient patients described had operations with no very excessive bleeding whereas in hemophilic patients operations are notoriously dangerous This observation if confirmed for other cases of factor VII deficiency suggests that the intrinsic (blood) thromboplastin system is the more important for sustained hemostasis This conclusion is also borne out by a more detailed study of hemophilic bleeding Hemophilic patients are very liable to secondary hemorrhage Even a treated patient will start to bleed if the treatment is inadequately maintained One large dose of AHG to a hemophilic patient will stop bleeding for 24 to 48 hr This observation suggests that normal hemostasis involves the continued renewal of clot and that the intrinsic thromboplastin mechanism is of primary importance for the process

If bleeding at different sites is considered it becomes clear that hemarthroses are associated with severe hemostatic defects whatever the cause Hemarthroses are usually linked with hemophilia because hemophilia is the most common serious hemostatic defect but mildly affected hemophilic patients do not have hemarthroses and severely affected patients with extrinsic thromboplastin defects do Hematuria can occur in any defect Persistent oozing from small skin injuries and from the gums is predominantly a feature of capillary abnormalities Deep muscle hematomas are much more common in hemophilia than in any other disease It may be that the muscle tissue is a poor source of thromboplastin and that muscle hemostasis normally depends very little on the extrinsic system The peculiar observation of Ackroyd that a factor VII-deficient patient tended to have recurrent hemorrhage from trivial superficial injuries and the capillary defect (indicated by a long bleeding time) which may occur in patients with vitamin K deficiency and after excessive dosage of dicoumarol suggest that the extrinsic thromboplastin system may have something to do with the maintenance of capillary function

There are certain interesting inconsistencies between laboratory results and hemostatic function The most obvious of these is the absence of

abnormal bleeding in patients with the Hageman defect. Workers are so familiar with the usual dire significance of a long clotting time that a syndrome in which abnormality in this test does not herald a severe hemostatic defect is a surprise. The only reasonable interpretation is that in the body the stimulus initiating coagulation is not a surface which resembles glass in any way. The glass surface may perhaps by-pass or replace the natural stimulus to clotting. The failure of the glass mechanism would not necessarily be associated with any in vitro hemostatic defect. One might reason that it should be possible to find patients who have clinical hemorrhagic states but in whom the natural stimulus to clotting is defective. Such patients should have no clotting abnormality if their blood is tested in glass tubes. Coagulation specialists often encounter patients who give a definite history of bleeding but whose laboratory tests are negative. Some of these cases may have the postulated defect, but proof is at present impossible.

Another and much more fundamental difficulty concerns the coagulation defect in afibrinogenemic patients. The bleeding tendency in these patients should be the most severe of all. In fact, the bleeding is usually less pronounced than in a severely affected hemophilic patient. This is a disturbing paradox to which at present there is no solution. It is difficult to understand why slow and defective clotting should be more dangerous than no clotting at all. Sooner or later this problem must be faced. It is possible, for example, that AHG is an essential component for healing and that one of the main defects in hemophilia is a delay in healing. It is also possible that the congenital absence of fibrinogen stimulates a particularly good mechanism of vascular contraction and platelet agglutination and that this mechanism can to some extent replace the functions of coagulation.

*Another perplexing problem concerns the different types of bleeding* in patients with hemophilia and von Willebrand's disease. Although patients with von Willebrand's disease may have low AHG levels, they do not bleed in the same way as hemophilic patients. The patients with von Willebrand's disease have epistaxis and the females have menorrhagia; they bruise easily but do not have massive muscle hematomas. Bleeding after dental extraction is immediate and readily controlled by pressure. Postoperative hemorrhage is rarely disastrous. The *characteristic hemophilic hemorrhage is a slow*, persistent ooze not controlled by pressure. Hemophilic patients do have deep muscle hematomas and postoperative bleeding is often disastrous. There is at present no explanation for this discrepancy. It is possible, of course, that the AHG deficiency of von Willebrand's disease is due to a rapid utilization of AHG by poor capillaries, and it is also possible that hemophilic patients often have some as yet ill-defined inhibitory factor which reinforces the underlying AHG defect.

## SUMMARY

1 Interpreted as molecular diseases the coagulation defects are sadly lacking in definition There is no biochemical definition by current techniques but only alignment with empirical schemata There is also no definition from clinical or metabolic features of the patients

2 In due course it may be possible to interpret coagulation defects in terms of deficiency of definite chemical species or reaction sequences At present no single chemical substance (except calcium) has been identified The present study of the role of phospholipids is of particular importance by continuing in this direction it may become possible for the first time to define a coagulation factor chemically

3 Complex events precede the formation of fibrin from fibrinogen This reaction is controlled by thrombin which in turn is derived from prothrombin by the combined action of calcium and plasma thromboplastin or prothrombinase

4 Prothrombinase activity resides either in an external system composed of tissue extract and calcium or in an internal system derived from the platelets plasma coagulation factors and $Ca^{++}$ Each of these prothrombinase systems is complex in itself and contains several factors necessary for prothrombinase activity

5 Clinical hereditary coagulation defects may result from deficiency of any one of the several components of the clotting mechanism Afibrinogenemia is one of these with mild clinical implications It is an autosomal recessive disorder

6 Defects in the extrinsic thromboplastin system include (*a*) factor V deficiency disease which may be a severe bleeding disorder and which has an uncertain inheritance pattern (*b*) factor VII deficiency also with severe bleeding tendency and probably autosomal recessive inheritance (*c*) Stuart Prower defect a disorder with clinical findings identical to those of factor VII deficiency and with autosomal recessive inheritance

7 Defects of the intrinsic thromboplastin system include (*a*) classical hemophilia which is a result of hereditary absence of the AHG component and has recessive sex linked inheritance (*b*) Christmas disease resulting from deficiency of plasma thromboplastin component indistinguishable clinically from classical hemophilia and having also a sex linked recessive inheritance (*c*) Hageman factor defect which has no clinical manifestations and (*d*) plasma thromboplastin antecedent a mild bleeding disorder with an uncertain inheritance pattern

8 Occasionally multiple factor defects are encountered Among these is von Willebrand's disease which may appear as a dual defect of the capillaries and the clotting mechanism The bleeding tendency is mild It is usually inherited as an autosomal dominant trait

9 No clarity has emerged from the study of blood coagulation factors

in relation to hemostasis but the shadowy outline emerging suggests that hemostasis may be achieved through several partly independent but connected mechanisms If one mechanism fails, others may in part replace it

10 At present it must be admitted that there is an atmosphere of fantasy and improbability about the whole subject Future workers may well view the present elaborate superstructure of hypothetical factors and hypothetically interpreted tests with the tolerance which we now afford to those who thought blood letting was a valuable therapeutic agency

## BIBLIOGRAPHY


1 Biggs R and Macfarlane R G *Human Blood Coagulation and Its Disorders* 2d ed Blackwell Oxford 1957
2 Biggs R Douglas A S and Macfarlane R G The action of thromboplastic substances J Physiol 122 554 1953
3 Hardisty R M The reaction of blood coagulation factors with brain extract Brit J Haematol 1 323 1955
4 *Owren P A Rapaport S I Hjort P and Aas K The biochemistry of thromboplastin its formation and action Sang 25, 752 1954*
5 Macfarlane R G A boy with no fibrinogen Lancet 1 309 1938
6 Fernando P B and Dharmasena B D A case of congenital afibrinogenemia Blood 12 474 1957
7 Gitlin D and Borges W H Studies on metabolism of fibrinogen in two patients with congenital fibrinogenemia Blood 8 679 1953
8 Lawson H A Congenital afibrinogenemia report of a case New England J Med *248 552 1953*
9 Graham J B Biochemical genetics of blood coagulation Am J Human Genet 8 63 1956
10 Biggs R and Douglas A S The measurement of prothrombin in plasma J Clin Path 6 15 1953
11 Quick A J Pisciotta A V and Hussey C V Congenital hypoprothrombinaemic states A M A Arch Int Med 95 2 1955
12 Quick A J The prothrombin in hemophilia and obstructive jaundice J Biol Chem *109 lxxiii 1935*
13 Owren P A The coagulation of the blood Investigation of a new clotting factor Acta med scandinav Suppl 194 1947
14 Lewis L H and Ferguson J H Hypoaccelerinemia Blood 10 351 1955
15 DeVries A Matoth Y and Shamir Z Familial congenital labile factor deficiency with syndactylism investigation on mode of action of labile factor Acta Haemat 5 129 1951
16 Alexander B and Goldstein R Parahemophilia in three siblings (Owren's disease) with studies on certain plasma components *affecting prothrombin* conversion Am J Med 13 255 1952
17 Iversen T and Bastings-Madsen P Congenital familial deficiency of factor V (parahemophilia) combined with deficiency of antihemophilic globulin Brit J Haemat 2 265 1956
18 Ratnoff O D Hereditary defects in clotting mechanisms in *Advances in Internal Medicine* vol IX Year Book Publishers Inc Chicago 1958
19 Hicks N D A coagulation disorder due to a factor VII like defect M J Australia 2 331 1955
20 Ackroyd J F The function of factor VII Brit J Haemat 2 397 1956
21 Pitney W R Congenital deficiency of factor VII Australasian Ann Med 7 15 1958
22 Voss D and Waaler B A Congenital hypoproconvertinemia A report on 12 cases with total deficiency and 19 cases with partial deficiency Thromb Diath Haem 3 375 1959

2a Dische F E and Benfield V Congenital factor VII deficiency Haematological and genetic aspects Acta Haemat 21 257 1959
3 Bergsagel D E *Stages in the Formation of Blood Thromboplastin* Thesis for the degree of Ph D University of Oxford 19 5
4 Telfer T P Denson K W and Wright D R A new coagulation defect Brit J Haemat 2 308 1956
25 Hougie C Barrow E M and Graham J B Stuart clotting defect I Segregation of an hereditary hemorrhagic state from the heterogeneous group heretofore called stable factor (S P C A proconvertin factor VII) deficiency J Clin Invest 36 485 1957
26 Graham J B Barrow E M and Hougie C Stuart clotting defect II Genetic aspects of a new hemorrhagic state J Clin Invest 36 497 1957
27 Bachmann F Duckert F Geiger M Baer P and Koller F Differentiation of the factor VII complex Studies on the Stuart Prower factor Thromb Diath Haem 1 169 1957
8 Denson K W Electrophoretic studies of the Prower factor a blood coagulation factor which differs from factor VII Brit J Haemat 4 313 1958
29 Roos J Van Arkel C Verloop M C and Jordan F L J A new family with Stuart Prower deficiency Thromb Diath Haem 3 59 1959
30 Tocantins L M Demonstration of antithromboplastic activity in normal and hemophilic plasma Am J Physiol 139 265 1943
31 Nour Eldin F and Wilkinson J F Bridge anticoagulant—a hitherto unrecognized blood clotting inhibitor in haemophilic and Christmas disease plasma Brit J Haemat 4 38 1958
32 Biggs R Assay of antihaemophilic globulin in the treatment of haemophilic patients Lancet 2 311 1957
33 Douglas A S Antihaemophilic globulin assay following plasma infusions in haemophilia J Lab Clin Med 51 850 1958
34 Blombäck M and Nilsson I M Treatment of hemophilia A with human antihemophilic globulin Acta med scandinav 161 301 1958
35 Hardisty R M and Pinniger J L Congenital afibrinogenaemia further observations on the blood coagulation mechanism Brit J Haemat 2 139 1956
36 Merskey C Occurrence of haemophilia in a human female Quart J Med 20 299 1951
37 Israels M C G Lempert H and Bilbertson E Hemophilia in a female Lancet 1 1375 1951
38 Quick A J and Hussey C V Hemophilia like states in girls Lancet 1 1234 1958
39 Brinkhous K M and Graham J B Hemophilia in female dogs Science 111 23 1950
40 Biggs R and Macfarlane R G Haemophilia and related conditions a survey of 187 cases Brit J Haemat 4 1 1958
41 Aggeler P M White S G Glendening M B Page E P Leake T B and Bates G Plasma thromboplastin component (P T C ) deficiency A new disease resembling haemophilia Proc Soc Exper Biol & Med 79 69 1952
42 Biggs R Douglas A S Macfarlane R G Dacie J V Pitney W R Merskey C and O Brien J R Christmas disease a condition previously mistaken for haemophilia Brit M J 2 1378 1952
43 Moor-Jankowski J K Huser H J and Truog G Der Bluterstamm von Tenna und seine Nachkommen 1650–1955 Acta genet et statist med 7 597 1958
44 Rosin S Moor-Jankowski J K and Schneeberger M Die Fertilität im Bluterstamm von Tenna (Hämophilie B) Acta genet et statist med 8 1 1958
45 Biggs R Sharp A A Margolis J Hardisty R M Stewart J and Davidson W M Defects in the early stages of blood coagulation a report of four cases Brit J Haemat 4 177 1958
46 Rosenthal R L Dreskin O H and Rosenthal N New hemophilia like disease caused by deficiency of a third plasma thromboplastin factor Proc Soc Exper Biol & Med 82 171 1953
4 Ratnoff O D and Colopy J E A familial hemorrhagic trait associated with a deficiency of a clot promoting fraction of plasma J Clin Invest 34 602 1955

48 Margolis J Initiation of blood coagulation by glass and related surfaces J Physiol 137 95 1957
49 Caen J and Bernard J Déficit en facteur Hageman Rev hémat 13 154 1958
50 Larrieu M J Soulier J P and Culot Y Déficit en facteur Hageman Sang 28 152 1957
51 Vreeken D Hoorweg P G and Dekker M A A case of serious clotting defect without hemorrhagic diathesis caused by P T A deficiency Nederl tijdschr geneesk 101 582 1957
52 Willebrand E A von Über hereditäre Pseudohaemophilie Acta med scandinav 76 52 1931
53 Nilsson I M Blomback M and Francken I von On an inherited autosomal hemorrhagic diathesis with antihemophilic globulin deficiency and prolonged bleeding time Acta med scandinav 159 35 1957
54 Pitney W R Personal communication
55 Biggs R Some observations on the blood of patients with factor VII deficiency Brit J Haemat 2 412 1956
56 Greig H B W and Tattersall J C Evidence for the existence of a third serum clotting factor Brit J Haemat 2 421 1956
57 Oeri J Matter M Isenschmid H Hauser F and Koller F Angeborener Mangel an Factor V (Parahaemophilie) verbunden mit echter Haemophilie A bei zwei Brudern Ann pædiat Suppl 58 1954
58 Seibert R H Margolius A and Ratnoff O D Observations on hemophilia parahemophilia and coexistent hemophilia and parahemophilia J Lab Clin Med 52 449 1958
59 Soulier J P and Larrieu M J Differentiation of hemophilia into two groups a study of 33 cases New England J Med 249 547 1953
60 Hill J M and Spear R J Combined hemophilia and P T C deficiency Blood 10 357 1955
61 Verstraete M and Vandenbrouche J Combined antihaemophilic globulin and Christmas factor deficiency in haemophilia Brit M J 2 1533 1955
62 Biggs R and Douglas A S The thromboplastin generation test J Clin Path 6 23 1953
63 Folch J Brain cephalin a mixture of phosphatides Separation from it of phosphatidyl serine phosphatidyl ethanolamine and a fraction containing inositol phosphatide J Biol Chem 146 35 1942
64 Biggs R and Bidwell E An attempt to identify a single phospholipid active in blood coagulation Brit J Haemat 3 387 1957
65 Poole J C F and Robinson D S Further observations on the effects of ethanolamine phosphatide on plasma coagulation Quart J Exper Physiol 41 295 1956
66 Robinson D S and Poole J C F The similar effect of chylomicra and ethanolamine phosphatide on the generation of thrombin during coagulation Quart J Exper Physiol 41 36 1956
67 Spaet T M Communication to the Discussion of Symposium X 4th International Congress of Biochemistry Vienna 1958
68 Therriault D Nichols T and Jensen H Purification and identification of brain phospholipids associated with thromboplastic activity J Biol Chem 233 1061 1958
69 Bell W N and Alton H G A brain extract as a substitute for platelet suspensions in the thromboplastin generation test Nature London 174 880 1954
70 Biggs R Douglas A S and Macfarlane R G The initial stages of blood coagulation J Physiol 122 538 1953
71 Bergsagel D E and Hougie C Intermediate stages in the formation of blood thromboplastin Brit J Haemat 2 113 1956
72 Biggs R and Bidwell E The kinetics of blood thromboplastin formation Communication to Symposium X 4th International Congress of Biochemistry Vienna 1958
73 Macfarlane R G Critical review the mechanism of haemostasis Quart J Med 33 1 1941

Part Ten

# Diseases Manifest Primarily in Renal Tubular Transport

---

# Chapter 36

# Familial Hypophosphatemia and Vitamin D-resistant Rickets

*T Franklin Williams Robert W Winters and Charles H Burnett*

## DEFINITION

Familial hypophosphatemia and vitamin D-resistant rickets may be defined as a specific disorder characterized by (1) hypophosphatemia associated with decreased renal tubular reabsorption of inorganic phosphate (2) familial occurrence the mode of inheritance being most probably sex linked dominant (3) the presence in some but not all affected persons of rickets or osteomalacia which does not respond to the usual doses of vitamin D (4) the absence of other related abnormalities

This definition excludes conditions which *may* share the same clinical or physiologic features or biochemical defects such as sporadic (non familial) vitamin D-resistant rickets associated with hypophosphatemia and familial vitamin D-resistant rickets associated with normal or high serum concentrations of inorganic phosphate Patients with multiple renal tubular defects such as the Fanconi syndrome or renal tubular acidosis appear to have similar but more extensive abnormalities

Regarding the limited group defined above as a separate entity has several advantages The clear-cut genetic pattern allows one to identify a substantial number of persons who presumably have the same fundamental physiologic and biochemical abnormality Furthermore the presence of a single gross defect in renal tubular function simplifies the analysis Clarification of a single aberrant mechanism is a logical step in the study of the multiple defects of related conditions

## HISTORICAL DEVELOPMENT

The growth of understanding of this disorder has proceeded along clinical physiologic and genetic pathways Rickets which failed to

respond to usual doses of vitamin D was recognized only after vitamin D–deficiency rickets had been largely eliminated by prophylaxis In such a patient Albright Butler and Bloomberg [1] in 1937 first showed that very large doses of vitamin D would bring about healing of the rickets That the hypophosphatemia was due either primarily or secondarily to decreased renal tubular reabsorption of phosphate was first recognized by Robertson Harris and McCune [2] in 1942

The first description of familial occurrence of vitamin D-resistant rickets with hypophosphatemia was in 1941 by Christensen [3] who reported a mother and her son and daughter with typical features of the disease Earlier instances of familial occurrence are probable [4] On the basis of skeletal manifestations several workers proposed an auto omal dominant mode of inheritance with variable penetrance [5 6] However Winters and his collaborators [7–9] were able to show that the occurrence of hypophosphatemia in involved families follows a sex linked dominant mode of inheritance with virtually complete penetrance They worked on the assumptions that the basic disorder lies in the abnormal renal excretion of inorganic phosphate with resulting hypopho phatemia and that the rickets and osteomalacia are secondary manifestations

## CLINICAL AND RADIOLOGIC FINDINGS

*The mildest abnormality is purely biochemical This is hypophosphatemia* (see below under Hypopho phatemia for criteria for hypopho phatemia) without clinical manifestations other than a slight decrease in height when compared with normophosphatemic siblings This is the most common finding in kindreds with the di order [7–9]

In hypopho phatemic adults the varying degrees of deformities due to rickets in childhood constitute more serious disturbances These include bowing of the legs and shortening of stature usually without evidence of continuing active bone di ease A few affected adults have evidence of *continuing osteomalacia* as judged by the presence of pseudofractures and an elevated erum alkaline phosphatase level These latter changes revert towards normal upon administration of massive doses of vitamin D

In affected children with rickets the disease is usually first recognized when the child begins to walk but the history or x ray examination often reveals abnormalities dating to the first year of life such as deformities of the skull late dentition and 'sitting' deformities of the legs These children usually have received prophylactic doses of vitamin D and have failed to respond to the doses given to cure what was initially thought to be vitamin D–deficiency rickets Healing of the active rickets occurs upon treatment with very large doses of vitamin D (usually 100 000 I U or more per day) Permanent deformities and shortened stature often are present In adults and in children the use of the massive doses of vitamin D required for healing frequently results in the appearance of

vitamin D intoxication with discontinuance of the vitamin the rickets usually becomes active again Thus vitamin D–resistant rickets differs clinically from vitamin D-deficiency rickets both in the hundredfold greater dose required to induce healing and in the fact that a permanent cure is rarely achieved until growth is completed

Among family members with hypophosphatemia females show considerably less bone disease than males Very few patients have the



Fig 36-1 Anteroposterior view of the knees of a 4 year-old boy (VI ) of I kindred of Winters et al [8]) showing marked rachitic changes with early healing and lateral curvature of femora and tibiae

muscular weakness and atony that are such prominent and frequent features of vitamin D–deficiency rickets Only one case of familial vitamin D-resistant rickets with findings suggestive of tetany has been reported [10] There has been no steatorrhea liver disease or renal disease other than the specific abnormalities discussed in the next section Craniostenosis and convulsions in infancy have occurred often enough in association with familial vitamin D–resistant rickets to raise the possibility of relationships between them

The radiologic findings in this syndrome are the same as those seen in rickets or osteomalacia from other causes In children with rickets the typical changes in the epiphyseal regions of the long bones are the most common and characteristic findings (Figs 36-1 36 2) Fractures and

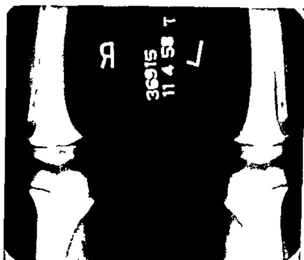



Fig 36-2 View of the knees of same patient as that in Fig 36-1 after 3 years of treatment with large doses of vitamin D Healing is still not complete



Fig 36-3 Anteroposterior view of the humerus of a 58-year-old man (IV-5 of the E kindred of Winters et al [8]) showing coarse trabeculation and a bony protuberance at the site of insertion of the deltoid muscle

pseudofractures and deformities of the skull thorax and long bones are also seen Additional changes which are occasionally present are similar to those seen in certain osteochondrodystrophies such as coarsened trabecular patterns in long bones cystic appearing areas in metaphyses and epiphyses and shortening and broadening of the long bones Occasionally abnormal bony protuberances appear at the site of major




Fig 36-4 Anteroposterior view of femur of a 41 year-old man (V 1⁹ of E kindred of Winters et al [8]) Note coxa vara lateral curvature of femur and pseudofracture of the femoral neck

muscular attachments and around the joints (Fig 36 3) In adults post rachitic deformities and rarely pseudofractures occur (Fig 36-4)

Table 36 1 summarizes schematically the principal clinical findings of this disorder The original reports of familial instances may be consulted for additional details [3 5–29a]

## HYPOPHOSPHATEMIA AND THE ABNORMALITY IN RENAL EXCRETION OF PHOSPHATE

### HYPOPHOSPHATEMIA

The significant place which a low serum concentration of inorganic phosphate occupies in this disease is high lighted by two lines of evidence

TABLE 36-1 SUMMARY OF FINDINGS IN FAMILIAL HYPOPHOSPHATEMIA AND VITAMIN D-RESISTANT RICKETS

| Type of hypophosphatemia | Age at onset, yr | Clinical and radiologic abnormalities | Concentration in serum | | | Other observations |
|---|---|---|---|---|---|---|
| | | | Calcium | Inorganic phosphate | Alkaline phosphatase | |
| 1 Asymptomatic hypophosphatemia | Under 1 | Slightly shortened stature | Normal | Low | Normal | No other abnormalities |
| 2 Hypophosphatemia in adults with *inactive postrachitic* deformities | Under 1 | Lateral (and usually anteroposterior) bowing of legs, shortened stature, occasionally coarsened trabeculation, rarefied areas | Normal | Low | Normal or slightly high | More frequent among hypophosphatemic males |
| 3 Hypophosphatemia in adults with deformities and active osteomalacia | Under 1 | Same as for (2) plus pseudofractures | Normal | Low | Slightly high | |
| 4 Hypophosphatemia with resistant rickets in childhood | Under 1 | Active rickets, occasionally coarsened trabeculation, shortened stature | Normal or slightly low | Low | High | More severe in affected males, craniostenosis and convulsions in a few instances |

(1) almost all cases which would be called resistant rickets on other grounds have also had hypophosphatemia (the normal values for serum phosphorus reported by Linnerloth et al [22] in cases with similar clinical features may have been obtained during treatment or those cases may represent a slightly different entity) (2) the probable mode of inheritance has been best revealed by using hypophosphatemia as the discriminant (*see below*)

In designating a person as hypophosphatemic, due allowance must be made for the normal changes in serum phosphorus with age and for differences between the sexes This was done by Winters and coworkers [7-9] who expressed mathematically and graphically and with limits of statistical confidence the relationship between serum phosphorus and age for each sex for a large number of observations on normal persons [30]

The age of first appearance of hypophosphatemia has not yet been determined The present authors have found the detection of hypophosphatemia in a young infant to be difficult even though at a later time the child proves to have the disease Whether or not this indicates some fundamental age conditioned difference in renal excretion of phosphate or other factors controlling the serum phosphorus in affected individuals is not known

Among hypophosphatemic individuals in affected families females have slightly higher values for serum phosphorus than males in the same family at all ages

The alkaline phosphatase level is elevated in affected persons with active rickets or osteomalacia Treatment with sufficient vitamin D to produce healing of the rickets is accompanied by a return of the alkaline phosphatase to or toward normal In some instances the serum phosphorus level has risen with treatment but healing has also been observed with no significant rise in phosphate concentration (see Treatment further on)

Other related serum chemical determinations usually give normal results The serum calcium concentration is normal or rarely slightly low Serum total protein albumin and globulin fractions urea or non protein nitrogen amino acids sodium potassium chloride carbon dioxide content and pH are uniformly normal

In every instance in which it has been studied the hypophosphatemia has been associated with increased renal excretion and decreased renal tubular reabsorption of phosphate This renal abnormality whatever its origin appears to be the principal cause of the hypophosphatemia Discussion of this abnormal function requires description of the normal mechanism of phosphate excretion and of factors known to influence it

## NORMAL MECHANISM FOR RENAL EXCRETION OF PHOSPHATE

The following points have been clearly established

1 At plasma concentrations up to at least 17 mg per 100 ml all the inorganic phosphate of the plasma is ultrafiltrable [31–33] The phosphate enters the glomerular fluid at virtually the same concentration as that in the plasma [34 35] Accordingly, the amount of phosphate filtered per unit time is the product of the glomerular filtration rate (as measured by clearance of an agent like inulin) and the plasma concentration of phosphate

2 In man and dog the net effect of tubular action on glomerular fluid is reabsorption of phosphate the amount of phosphate excreted is normally only a small fraction of that filtered The evidence obtained in the dog by Pitts et al [36] using the stop flow technique suggests that reabsorption of phosphate occurs in the proximal tubule Only in rare instances [37–40] has evidence for net tubular secretion of inorganic phosphate in man been found Net tubular secretion of inorganic phosphate has been demonstrated in chickens [41] in cats after infusion of organic phosphates [42] and probably in acidotic dogs [42a]

3 In man and the normal dog a maximal rate of net tubular reabsorption of inorganic phosphate ($Tm_P$) is readily demonstrated If the concentration of phosphate in the plasma is raised progressively the calculated rate of net reabsorption of phosphate (phosphate filtered minus phosphate excreted) reaches a constant value and further increments in filtered phosphate are accompanied by a corresponding increase in excreted phosphate When measured under standardized conditions the values for $Tm_P$ are reasonably constant in normal individuals on normal diets Most reported normal values for $Tm_P$ for children and adults are between 120 and 150 $\mu$m phosphate reabsorbed per 100 ml glomerular filtrate although a few supposedly normal individuals have had values as low as 80 $\mu$m per 100 ml glomerular filtrate [43–49] These results do not exclude tubular secretion of phosphate but they do indicate that if secretion occurs it must be at a constant rate and must be unaffected by progressive rises in plasma phosphate concentration

The $Tm_P$ is the most meaningful physiologic measurement of renal tubular activity in any consideration of phosphate excretion Other appraisals of the renal mechanism for excretion of phosphate have frequently been used Among these are the renal clearance of phosphate ($C_{PO}$ = excreted phosphate/serum phosphorus) the percentage of filtered phosphate that is reabsorbed (%TRP) and the fraction of filtered phosphate that is not reabsorbed (= $C_{PO}/C_I$ ) These estimations are made without loading the excretory mechanism with phosphate Since these three calculations are all influenced by the serum phosphate concentration as well as by glomerular and tubular function direct interpretation of

tubular activity from them is extremely difficult Their use has at times led to confusing and erroneous views In patient IV 5 of Table 36-3 for example before phosphate loading the $C_{PO}$ was 13 ml per min and the %TRP was 87 both within the range of 'normal' yet the $Tm_P$ was distinctly low (This patient had normal intake of phosphate prior to these measurements an unusually high or low intake will be reflected in changes in $C_{PO}$ %TRP and $C_{PO}/C_I$ because of the effect on serum phosphate concentration without any change necessarily in tubular function) As suggested by Lambert et al [46] and Milne et al [50] care should be taken to compare these calculated values with those obtained for normal subjects at the same level of serum phosphorus *When the glomerular filtration rate is normal* (see below) it may be possible thus to separate abnormal from normal phosphate excretory patterns In the example cited above a $C_{PO}$ of 13 ml per min is actually probably high for a serum phosphorus of 2 4 mg per 100 ml

In the presence of renal disease with significant reduction in glomerular filtration rate the (unloaded) tubular reabsorption of phosphate is usually depressed out of proportion to the decrease in glomerular filtration [51] It is clear that interpretations of tubular reabsorption of phosphate in other abnormal states cannot be made when there is significant concomitant reduction of glomerular filtration This change in tubular reabsorption may conceivably be related to increased parathyroid function (*see* Hormonal Factors below)

## FACTORS KNOWN OR THOUGHT TO INFLUENCE THE NORMAL MECHANISM FOR EXCRETION OF PHOSPHATE

### *Possible Effects of Acute Changes in Glomerular Filtration Rate*

In the dog changing the glomerular filtration rate—by varying the protein intake—causes no change in $Tm_P$ [52] Evidence in man is less clear-cut partly because of the difficulty of producing large changes in glomerular filtration rate [45 47] Gross abnormalities of tubular reabsorption as seen for example in hyperparathyroidism and familial hypophosphatemia may occur without any evidence of changes in glomerular filtration rate [8 49 53]

### *Hormonal Factors*

Intramuscular administration of extracts of bovine parathyroid glands over several days to normal subjects and hypoparathyroid patients produces an increased excretion of phosphate a fall in serum phosphorus and a fall in $Tm_P$ [54] Patients with proved hyperparathyroidism have low values for $Tm_P$ and the $Tm_P$ eventually returns to normal or nearly normal after removal of the overactive parathyroid tissue [49 53 55] provided there has been no permanent renal damage Thus there is little

## NORMAL MECHANISM FOR RENAL EXCRETION OF PHOSPHATE

The following points have been clearly established

1 At plasma concentrations up to at least 17 mg per 100 ml all the inorganic phosphate of the plasma is ultrafiltrable [31–33] The phosphate enters the glomerular fluid at virtually the same concentration as that in the plasma [34, 35] Accordingly, the amount of phosphate filtered per unit time is the product of the glomerular filtration rate (as measured by clearance of an agent like inulin) and the plasma concentration of phosphate

2 In man and dog the net effect of tubular action on glomerular fluid is reabsorption of phosphate the amount of phosphate excreted is normally only a small fraction of that filtered The evidence obtained in the dog by Pitts et al [36] using the stop-flow technique suggests that reabsorption of phosphate occurs in the proximal tubule Only in rare instances [37–40] has evidence for net tubular secretion of inorganic phosphate in man been found Net tubular secretion of inorganic phosphate has been demonstrated in chickens [41] in cats after infusion of organic phosphates [42] and probably in acidotic dogs [42a]

3 In man and the normal dog a maximal rate of net tubular reabsorption of inorganic phosphate ($Tm_P$) is readily demonstrated If the concentration of phosphate in the plasma is raised progressively the calculated rate of net reabsorption of phosphate (phosphate filtered minus phosphate excreted) reaches a constant value and further increments in filtered phosphate are accompanied by a corresponding increase in excreted phosphate When measured under standardized conditions the values for $Tm_P$ are reasonably constant in normal individuals on normal diets Most reported normal values for $Tm_P$ for children and adults are between 120 and 150 μm phosphate reabsorbed per 100 ml glomerular filtrate although a few supposedly normal individuals have had values as low as 80 μm per 100 ml glomerular filtrate [43–49] These results do not exclude tubular secretion of phosphate but they do indicate that if secretion occurs it must be at a constant rate and must be unaffected by progressive rises in plasma phosphate concentration

The $Tm_P$ is the most meaningful physiologic measurement of renal tubular activity in any consideration of phosphate excretion Other appraisals of the renal mechanism for excretion of phosphate have frequently been used Among these are the renal clearance of phosphate ($C_{PO}$ = excreted phosphate/serum phosphorus) the percentage of filtered phosphate that is reabsorbed (%TRP) and the fraction of filtered phosphate that is not reabsorbed (= $C_{PO}/C_I$ ) These estimations are made without loading the excretory mechanism with phosphate Since these three calculations are all influenced by the serum phosphate concentration as well as by glomerular and tubular function direct interpretation of

on tubular reabsorption of phosphate are needed The evidence presented to date is that parathyroid hormone (which has many actions similar to those of the vitamin D group of sterols) cortisone possibly stilbestrol and possibly vitamin D under certain circumstances (see below) all decrease the tubular reabsorption of phosphate This suggests a common mechanism of action on the renal tubules The possibility that abnormal steroids may be involved in vitamin D-resistant rickets is discussed further on under Possible Mechanisms of Pathogenesis Other relationships between cortisone and vitamin D are discussed under Other Possible Mechanisms of Pathogenesis

*Chemical Substances Possibly Competing for Tubular Reabsorption of Phosphate*

A number of substances appear to compete with inorganic phosphate for renal tubular reabsorption Thus when the filtered load of any of these is increased and its rate of tubular reabsorption increases the tubular maximum for reabsorption of phosphate is simultaneously depressed Glucose [72 73] acetoacetate [74] bicarbonate [75] glycine and alanine [52] are known to have this effect It seems probable that glucose and acetoacetate affect phosphate reabsorption through a mechanism different from that of amino acids and bicarbonate No direct studies of the simultaneous effects of these agents have been made Phlorizin in doses which inhibit or block tubular reabsorption of glucose *increases* the tubular reabsorption of phosphate This suggests that even the normal rate of reabsorption of glucose has a slightly suppressive effect on phosphate reabsorption [72 73] The carbonic anhydrase inhibitor acetazolamide depresses the $Tm_P$ concomitant with increasing bicarbonate excretion [75 76]

The interrelationships between the pathways for tubular reabsorption of these substances are quite complex and in need of much further study Lotspeich discusses this problem in relation to the hypothetical biochemical pathway which actively transfers phosphate across the tubular cell membrane [76a] The inference that the inhibition may be of a competitive type is pertinent to the decreased tubular reabsorption of phosphate in vitamin D-resistant rickets The occasional appearance of slight glucosuria and aminoaciduria in this disorder is compatible with possible abnormalities in the metabolism of these agents in the production of the phosphaturia These interrelationships are discussed in more detail in Chap 37

There is equivocal evidence concerning the effects of hypokalemia on the tubular reabsorption of phosphate Hogben and Bollman [32] found that potassium infusion produced a rise in $Tm_P$ but the effect was inconstant In one patient with potassium losing renal disease Mahler and Stanbury [77] found increased excretion of phosphate despite a low

doubt that one specific action of the parathyroid hormone is on renal tubular reabsorption of phosphate Earlier studies of this action are referred to by Bartter [44] and Lotspeich [56]

The effects of varying intakes of calcium and phosphate on renal tubular reabsorption of phosphate are probably mediated through the parathyroid glands Prolonged increased intake of phosphate results in a reduction in the amount of phosphate reabsorbed by the renal tubule [57] Prolonged elevation of serum phosphorus level by repeated infusions leads to a marked fall in $Tm_P$ in normal persons but probably not in hypoparathyroid patients [58] A low intake of calcium produces parathyroid hyperplasia in animals [59 60] Secondary hyperplasia of the parathyroid glands in patients with renal disease is associated either with decreased total calcium concentration in the serum [61] (and decreased ionized calcium of the serum [62]) or with elevated serum phosphorus concentration or both Calcium infusion is usually followed by a temporary increase in tubular reabsorption of phosphate (unloaded) and in $Tm_P$ [63–66] All these findings are best interpreted as indicating that a low calcium intake or absorption or a high phosphorus intake leads to parathyroid hyperplasia which in turn leads to decreased tubular reabsorption of phosphate

There is evidence that in the chicken parathyroid hormone increases the net tubular secretion of phosphate [41] Since tubular secretion of phosphate in man has not been excluded the possibility must be kept in mind that parathyroid hormone also affects tubular secretion in man

Certain other hormones or related compounds have been studied for their effects on renal phosphate excretion Cortisone given intravenously to dogs produces an immediate fall in $Tm_P$ but deoxycorticosterone does not [67 68] The evidence is less clear cut in man ACTH and cortisone given in large amounts for several days cause increased phosphate excretion because of a decrease in the net amount of phosphate reabsorbed [40] In one experiment the evidence suggested net tubular secretion In acute studies using intravenous hydrocortisone no change in $Tm_P$ in some normal subjects and a slightly decreased $Tm_P$ in others was found [69] A decrease was more frequent when $Tm_P$ was expressed per unit volume of glomerular filtrate [69] Diethylstilbestrol given for 14 to 21 days to five patients (three with osteoporosis) was followed by some decrease in $Tm_P$ in each [70] One cannot say from these data whether stilbestrol has a direct action on the renal tubules

Triiodothyronine given intravenously to dogs in 1 mg doses induced increased renal excretion of phosphate and it was stated that tubular reabsorption was decreased [71] However serum phosphorus level and glomerular filtration rate rose and in the published data the calculated rate of tubular reabsorption actually increased

Further studies of the action of these hormones and related compounds

compared with the $Tm_P$ of a previous day The data are included in Table 36 4 Glomerular filtration rate did not change Huge doses of vitamin D given to normal dogs (1 25 to 3 mg per kg given over 5 days) depress glomerular filtration rate as well as $Tm_P$ but the fall in $Tm_P$ is out of proportion to the decline in glomerular filtration rate [88]

These results may represent an action of massive doses of vitamin D and related compounds which is shared with other sterols and which is different from the physiologic effects of vitamin D on the kidney

Vitamin D in large doses raises the serum concentration and excretion of citrate to abnormally high levels [89 90] Since infusing citrate into a hypoparathyroid subject does not produce increased phosphate excretion [80] whereas vitamin D has this effect in the same type of subject [84-86] it seems unlikely that vitamin D affects phosphate excretion through its effect on citrate metabolism More definitive studies of this point are needed

## THE RENAL ABNORMALITY IN PHOSPHATE EXCRETION

Beginning with the observations of Robertson Harris and McCune [?] in 1942 many studies of patients with vitamin D resistant rickets

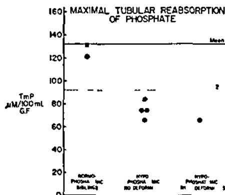



Fig 36-5 Values for maximal tubular reabsorption of pl o phate ($Tm$ $\mu$ moles per 100 ml gl m rular fltrate) on 10 members of the F kin lred of Winters et al ■ = male ● = female The mean an l two standard deviations ( ) for the valu s of $Tm_P$ of ?( normal subjects [+3 45 and unpubl she l observations of the authors] are represented by the lines

have shown abnormally high rates of excretion of phosphate or clearances of phosphate especially when considered with respect to their low values of serum phosphorus [3 5 17 21 23 29 87 91-93] This has been true both in cases with a clear-cut familial incidence [3 ?8 29 87] and in other cases lacking an obvious family history

Unequivocal demonstration of the nature of the principal renal abnormality in this di e e though not of its mechani m has come from

serum phosphorus level when the patient was hypokalemic Phosphate excretion and serum phosphorus level returned to normal when the potassium deficiency was corrected but other changes were going on simultaneously, such as the correction of a metabolic acidosis As noted above, deoxycorticosterone had no effect on $Tm_P$ in an experiment in which serum potassium concentration fell from 3 85 to 2 6 mEq per l [67]

Systemic acidosis is accompanied by decreased tubular reabsorption of phosphate in the nonloaded state in the human being [78], in the dog conflicting evidence has been presented [72 79] $Tm_P$ is not affected by acidosis in either species

Nordin and Fraser [80] found that infusion of sodium citrate led to increased phosphate excretion without significant change in serum phosphate concentration Glomerular filtration rate was not measured but if it did not change, then tubular reabsorption of phosphate was diminished This response did not occur in one hypoparathyroid subject and Nordin and Fraser suggested that the effect of citrate was mediated through the parathyroid gland i e, that the infused citrate formed a complex with the ionized calcium of serum, thereby lowering ionized calcium and stimulating parathyroid hormone secretion The rate of citrate excretion was not measured The direct role of citrate excretion if any, in phosphate excretion has not been evaluated

*Action of Vitamin D on Renal Tubular Function and Excretion of Phosphate*

Existing information does not permit an unequivocal statement concerning the effects of vitamin D on renal tubular function in normal or diseased states

Vitamin D given to vitamin D-deficient rachitic patients and dogs produces an *increased* tubular reabsorption of phosphate [81] and increased $Tm_P$ [82, 83] This result could be a direct effect of vitamin D on the renal tubule It might also be an indirect effect, with the well established action of vitamin D in increasing calcium absorption resulting in suppression of parathyroid function As Albright has pointed out [61] these two possibilities could be differentiated by repeating the study on rachitic dogs with and without parathyroidectomy, but this has not yet been done

In hypoparathyroid human subjects vitamin D in massive doses (equivalent to 5 to 10 mg calciferol a day—1 000 times the maintenance requirement of vitamin D) and dihydrotachysterol (A T 10) 6 to 12 mg per day have both produced significant increases in phosphate excretion concomitant with a falling serum phosphorus [84 86] Unless glomerular filtration rate changed these results indicate *decreased* tubular reabsorption of phosphate

Rupp and Swoboda [87] gave 20 mg of a vitamin $D_2$-hydrosol intravenously to one normal subject and found a slightly lower $Tm_P$ when

Table 36-3 Renal Tubular Reabsorption of Phosphate in Members of E I R D

| Subject generation and No. | Age, y | Sex | Height, In | Mean values before phosphate loading: $P_{PO}$ mg/100 ml | $P_{PO}$ mM/l | $C_I$ l/min | Exc P µM/min | Reabs P µM/100 ml GF | Mean values after phosphate loading: $C_I$ l/min | Reabs P µM/100 ml GF |
|---|---|---|---|---|---|---|---|---|---|---|
| A Hypophosphatemic patients with deformities | | | | | | | | | | |
| V 1 | 41 | M | 65 | 1 8 | 0 41 | 144 | 13 8 | 31 6 | 135 | 67 |
| V 1 † | | | | 1 96 | 0 63 | 131 | 18 5 | 60 3 | 170 | 81 |
| IV 5 | 58 | M | 58 | 2 48 | 0 80 | 98 | 10 6 | 59 2 | 101 | 54 |
| IV 5‡ | | | | 2 11 | 0 68 | | 3 6 | | 111 | 56 |
| IV 5§ | | | | 2 40 | 0 7 | 105 | 20 0 | 58 0 | 93 | 81 |
| V 15 | 3 | M | 67 | 1 8 | 0 58 | | | | 166 | 79 |
| V 13 | 8 | F | 62 | 2 20 | 0 71 | | | | 93 | 66 |
| B Hypophosphatemic patients with no deformities | | | | | | | | | | |
| V 3 | 33 | F | 64 | 2 48 | 0 80 | 112 | 0 5 | 59 7 | 120 | 84 |
| V 4 | [illegible] | F | 6 | 2 14 | 0 69 | | | | 101 | 75 |
| V 10 | 3 | F | 64 | 2 50 | 0 81 | | | | 92 | 66 |
| V 8 | | F | 6 | 40 | 0 78 | | | | 134 | 75 |
| C Normophosphatemic siblings | | | | | | | | | | |
| V 8 | | F | 68 | 3 00 | 0 97 | | | | 1 8 | 121 |
| V 5 | 31 | M | 71 | 3 54 | 1 14 | | | | 141 | 131 |

Column headings $P_{PO}$ = serum concentration of inorganic phosphorus $C_I$ = clearance of inulin L cr I = rate of phosphate excretion Reabs P = rate of phosphate reabsorption GF = glomerular filtrate

† After vitamin D 300 000 I U /day for 6 months

‡ After low-calcium diet plus Basaljel 180 ml/day for 7 days

§ Normal diet intravenous loading with calcium gluconate (13 mg calcium per kg over 6 hr) Values before phosphate loading were obtained at 4 to 5 hr and after phosphate loading at 5 to 6 hr after start of calcium infusion

Source R W Winters et al [6] and previously unpublished observations of the authors

TABLE 36-2 SUMMARY OF EVIDENCE ON PHOSPHATE EXCRETION IN PATIENTS WITH VITAMIN D-RESISTANT RICKETS RECALCULATED

| Auth and ifer | Ag wh t d d y | S | GFR ml ml/m | Ser m l mg/100 ml | Cp ml/m | % filtered sub- ptio f P µM/100 ml GFR | P loaded reab p- tio f P (Tmp) µM/100 ml GFR |
|---|---|---|---|---|---|---|---|
| A F m?lial i m t |  |  |  |  |  |  |  |
| Ch t e [3] | 15 | M |  | 2 | P e cret on *(a tl t (f n m l l ject on d t |  |  |
|  | 13 | F | Blood a ^ mg/ 100 ml | 0 | P cret fe that f n m l e bj ct on sa d t |  |  |
| T bl t al [29] | 1 | F | NPN 26 mg/100 l | 7 | 10 7† |  |  |
|  | 14 | F | NPN 9 mg/100 l | 4 | 19 † |  |  |
| F nd G a d t [17] ( —m r th n m(l D t e k ts) | fs | M | Ct * 99 | 0-3 3 | 7 9-16 4 |  |  |
| 2 mal b) t | 31 | F | BUN 14 g/100 l | 1 1 1 6 | 10 0 |  |  |
| R pp nd Sw t od [9] | 5 10 |  |  | 3 7 | 1 7 3 5 |  |  |
| Case 1 |  |  |  |  |  |  |  |
| Case 3 | 6 | M | C 0 | 0 |  |  |  |
| Case 5 | 9 | M | Ct 93 | 1 8 |  | 56 | 6 100 |
| N rm l | 5 | F | Ct 62 |  |  | 43 | 68 84 |
| Lamy t al [21] |  |  | Ct 111 | 4 3 |  | 6 | 100-140 |
| K d [102] | 13 | F | $C_{cre}$ ca 106 | 1 9 | 11 | 118 | 167 07 |
| B N f mily h t ry gi en | 6 8 (br tl rs) | M | N m l (ma t l) | N gl |  |  |  |
| Kl n a d Gow [104] |  |  |  |  |  |  |  |
| R berts n t l [2] | 5 | M | Ct 55 | 3 3 |  |  |  |
| T bl t al [ 9] | 10 | M |  | 2 6 | 8 8 |  | 84 99 |
|  | 4 | F | NPN 7 mg/100 ml | 2 ? | 9 1† |  |  |
|  |  |  | NPN 35 mg/100 l | 2 3 | 3 6 |  |  |
| Lambe t t al [40] 5 n l g l |  | F |  | 2 3 3 0 | 8 9 3 2 4 9 11 8 |  |  |

C = l a f l
† P 1 73 m rfac a a

arginine [97] or methionine [99] are probably abnormal in patients with vitamin D-resistant rickets but more specific information is needed on the disease of the subjects and on comparable results in normal persons

There is meager evidence for an association of renal glycosuria with vitamin D-resistant rickets The only patients described so far with this combination and with family histories of vitamin D-resistant rickets are the young boy reported by Fanconi and Girardet [17] and Tobler et al [29] who was unusual in several other ways and one of two brothers both of whom were found by Dent [91] to have vitamin D-resistant rickets Only rare instances of glycosuria have been reported among nonfamilial cases [100 101] The large body of negative evidence for renal glycosuria in patients with familial hypophosphatemia and vitamin D-resistant rickets suggests that an abnormality in glucose reabsorption does not participate in the production of the increased phosphate excretion Quantitative determinations of tubular maxima for glucose reabsorption in subjects with clear family histories are needed to clarify this point

The glomerular filtration rate has been normal in the patients in whom it has been measured (Tables 36-2 36-3) Freeman and Dunsky [100] also found a normal exogenous creatinine clearance in their patient Acidification of the urine is probably normal as shown by normal $CO_2$ content of the blood The only exceptions have been isolated low values for $CO_2$ content in the child reported by Fanconi and Girardet [17] and in one patient reported by Swoboda [28] In addition a few observers have found a normal response to an ammonium chloride load [21 94 102] Concentrating ability is normal as are concentration and dilution tests [17 21] There is no evidence of abnormalities in sodium or potassium excretion and serum concentrations are normal Renal response to administration of parathyroid hormone is probably normal (see below) Response to other agents like cortisone has not been determined There are no reports of more quantitative measures of renal function such as the tubular maxima for excretion of para aminohippurate

The renal abnormality in familial hypophosphatemia thus appears to involve solely the tubular reabsorption of phosphate More quantitative information is needed in certain areas in particular the response of subjects with clearly defined genetic backgrounds to loading with amino acids glucose and para aminohippurate An equally great need is for histologic and histochemical study of renal tissue Such information is entirely lacking

### EFFECTS OF VITAMIN D ON RENAL FUNCTION IN VITAMIN D-RESISTANT RICKETS

The renal effects of vitamin D in this disorder have been incompletely studied The administration of large doses of vitamin D causes either no

measurement of $Tm_P$ by Rupp and Swoboda [87] and by Winters et al [7, 8] In both studies the $Tm_P$ was distinctly low Tables 36 2 and 36 3 and Fig 36 5 present the available data The values for $Tm_P$ in members of the "E" kindred of Winters et al were about equally low in four adults with hypophosphatemia without bone disease and in four adults with hypophosphatemia and bone disease

The hypophosphatemia appears to be almost entirely due to decreased tubular reabsorption of phosphate Phosphate balance may be otherwise normal (see below) At sufficiently low serum phosphate levels such as when intake is very low phosphate balance is maintained with minimal phosphate excretion [94]

The possibility that extrarenal factors such as parathyroid hormone are involved in the pathogenesis of the decreased renal tubular reabsorption of phosphate is discussed below, under Possible Mechanisms of Pathogenesis

## OTHER RENAL ABNORMALITIES

Except for the low tubular reabsorption of phosphate renal function is normal as far as can be determined from present data The occurrence of slight degrees of aminoaciduria and glycosuria in a few cases suggests that in some instances of this disease there may be additional defects similar to those in Fanconi syndrome In attempting to evaluate the degree of association if any between familial hypophosphatemia and the Fanconi syndrome the study of families with well documented genetic patterns should prove particularly valuable In one of the families most carefully studied from a genetic viewpoint total α amino acid excretion in five hypophosphatemic persons was normal [8] Paper chromatograms of the amino acids of the urine of a number of affected adults and children in the families studied by these authors also gave normal results [95] All other determinations of aminoaciduria in familial instances of the disorder have been normal [13 21 28 29] except for one case first reported by Fanconi and Girardet [17] This child had slightly elevated serum amino acid concentration on one occasion and slightly increased urinary excretion of amino acids on three occasions The patient also had slight glycosuria [29] this coupled with unusual facies and an abnormal skull x ray led Tobler and coworkers to question whether he had more than simple vitamin D-resistant rickets [29]

Observations on aminoaciduria in patients with vitamin D-resistant rickets without family history have been more varied Some patients have had no abnormal aminoaciduria [25 29 96] In others abnormally high excretion of various amino acids has decreased upon administration of large doses of vitamin D [21 25 97] a response similar to that of the aminoaciduria of vitamin D-deficiency rickets to smaller doses [97 98] The excretory patterns of amino acids after loading with histidine-

excretion of these substances This response was recorded throughout two to six 6-day collection periods Since there were no measurements of glomerular filtration rate it is not known whether any change in tubular reabsorption of phosphate occurred If glomerular filtration rate did not change significantly then tubular reabsorption of phosphate must have decreased The authors proposed that this indeed did happen and may have been due to stimulation of parathyroid hormone by the fall in serum calcium concentration

TABLE 36-4 ACUTE EFFECT OF INTRAVENOUS VITAMIN D ON MAXIMAL TUBULAR REABSORPTION OF PHOSPHATE (*Tmp*)

| *Case No* | *Dose of vitamin* | *Range of Tmp* | | |
|---|---|---|---|---|
| | | *Before vitamin D* | *Immediately after vitamin D* | *1 week after vitamin D* |
| 5 (RR)† | 0 mg | 97–100 | 93–103 | 103–119 |
| 5 (RR) | 10 mg | 100–145‡ | 1 4 1[illegible] | |
| 3 (RR) | 10 mg × [illegible] | 68–84‡ | 110 1[illegible] | 5[illegible]8 |
| Normal | 10 mg × 2 | 174–208‡ | 145–148 | 1[illegible]–135 |
| 1 (RR) | | 65–100 | | [illegible]–1[illegible] |

Expressed in micromoles per 100 ml glomerular filtrate
† RR = resistant rickets
‡ These control measurements of *Tmp* were done on a previous day and do not represent control values immediately prior to injection of vitamin D
SOURCE Recalculated from Rupp and Swoboda [S ]

In summary large doses of vitamin D may result in increased renal tubular reabsorption of phosphate in patients with familial hypophosphatemia and vitamin D–resistant rickets Even if true this is not necessarily a direct action of vitamin D The proposal originally made by Albright et al [S4 S6] and subsequently by numerous students of this disease that the purported change in tubular reabsorption is the result of diminished parathyroid activity as calcium absorption improves has yet to be proved or disproved

## GASTROINTESTINAL ABSORPTION OF CALCIUM AND PHOSPHATE

### NORMAL FEATURES AND THE ROLE OF VITAMIN D

Normal adults and children supplied with physiologic amounts of vitamin D and dietary calcium above a minimal level (0 45 to 1 0 gm per day [10 10?]) absorb enough calcium to remain in calcium balance Children achieve enough positive balance for normal growth of bone A deficiency of vitamin D intake uniformly leads to progressively lowered absorption of calcium until fecal excretion of calcium equals intake

change or a rise in serum phosphorus level accompanied by either no change or a fall in urinary phosphate excretion [*1, 2, 5, 17, 94, 102*]. Whether these changes are the result of decreased glomerular filtration rate or increased renal tubular reabsorption of phosphate is not known and cannot be determined from measurement of phosphate clearance alone. The studies reported by Robinson and Nelson [*103*] in which $C_{PO}$ remained constant as serum phosphorus concentration rose with vitamin D therapy, offer an illustration of the care with which clearance data must be interpreted. If glomerular filtration rate remained constant, as inferred in four of six subjects, a constant $C_{PO}$ in association with a rising serum phosphorus concentration requires a simultaneous *increase* in both phosphorus reabsorption and excretion in these subjects. Only if there were a considerable fall in glomerular filtration rate could the results of Robinson and Nelson indicate no change or a decrease in tubular reabsorption of phosphate with vitamin D therapy.

There have been few direct measurements of glomerular filtration rate in relation to vitamin D therapy. In one subject stated to have familial vitamin D–resistant rickets, Klein and Gow [*104*] found a rise in inulin clearance one day after giving 600 000 units (22 mg) of vitamin D intramuscularly. Robinson and Nelson [*103*] described a fall in glomerular filtration rate in two of six subjects with vitamin D–resistant rickets on prolonged vitamin D therapy. In one subject with this disease reported by Dent and Harris [*5*], daily creatinine excretion did not change during vitamin D administration.

Rupp and Swoboda [*87*] have reported the acute response of phosphate excretion to intravenous vitamin D administration both in nonloaded and phosphate-loaded patients. They studied three children with familial vitamin D–resistant rickets. Glomerular filtration rate was little affected. The effects on $Tm_P$ are summarized in Table 36-4. In two patients $Tm_P$ was higher immediately after administration of vitamin D, and in one it did not change. There was no change in phosphate reabsorption in one subject studied without phosphate loading. These data cannot be considered conclusive evidence for a direct effect of vitamin D on phosphate reabsorption.

Measurements of $Tm_P$ and phosphate reabsorption in the nonloaded state 1 week after these acute intravenous doses were given were not different from control observations [*87*]. If vitamin D has an effect on renal function, it is short lived. In a preliminary report Dent has stated that there is a rise in $Tm_P$ with administration of vitamin D [*105*]. The route, amount, and duration were not specified.

Zetterstrom et al. [*106*] made the curious and thus far unique observation in two children with vitamin D–resistant rickets that the initial response to 100 000 to 200 000 I.U. per day orally was a fall in serum concentrations of phosphate and calcium without change in absorption or

in vitamin D-resistant rickets is furnished by the studies of Saville et al [114] and Fraser et al [119] These workers found that administration of large amounts of phosphate either orally or intravenously without large doses of vitamin D will induce increased positive balances of calcium and phosphorus and initiate healing of the rickets Studies of the absorption of calcium and phosphate in affected members of families with the best worked out pedigrees based on hypophosphatemia have not been made

In summary the gastrointestinal absorption of calcium and secondarily of phosphate is probably abnormally low This is particularly evident in children with active rickets who do not have a sufficiently positive balance for normal growth In affected family members with asymptomatic hypophosphatemia impairment in calcium and phosphate absorption may not be apparent Bony development is normal both clinically and radiologically Vitamin D in massive doses will induce increased absorption of calcium and phosphorus This is probably no different from what occurs in normal persons despite the lack of response to physiologic amounts of vitamin D Large increases in intake of phosphate also produce increased absorption of phosphate and probably of calcium

## ABNORMALITIES IN METABOLISM OF BONE

A thorough discussion of the normal mechanism of formation of bone and the pathogenesis of rickets due to deficiency of vitamin D is beyond the scope of this chapter good descriptions may be found in Eliot and Park [120] Neuman and Neuman [121] and Bourne [122]

In considering the bony abnormalities found in familial hypophosphatemia and vitamin D-resistant rickets it must be remembered that this inherited disorder can be manifested by hypophosphatemia and diminished tubular reabsorption of phosphate alone with *no apparent bone disease* Although no bone tissue has been examined histologically from such subjects there are no radiologic abnormalities The only clinically discernible abnormality is slightly shorter stature than that of normophosphatemic siblings [8] This virtual lack of bone disease despite hypophosphatemia indicates (1) that the genetically transmitted abnormality is not concerned directly with bone metabolism and (2) that factors other than simple hypophosphatemia are participating in those patients who develop bone disease

Two different types of bony abnormalities have been described in patients with vitamin D-resistant rickets The most characteristic finding and the one which gives the disease its name is the typically rachitic appearance of the epiphyseal region There is a markedly expanded zone of proliferating cartilage with increased osteoid tissue and invasion by wide tortuous blood vessels [1 16 28 94 123 124] The e

Urinary calcium excretion may become virtually zero Studies of animals and man on calcium free diets show that little if any of the fecal calcium is due to calcium excretion into the gastrointestinal tract [84 86, 110, 111] The administration of vitamin D to vitamin D-deficient subjects leads to an increase in calcium absorption [111–113] This action of vitamin D is the most clearly established role of the vitamin in maintaining normal metabolism of calcium and phosphate The mechanism of action has not been determined

Intestinal absorption of phosphate is probably not directly dependent upon vitamin D [110] In vitamin D-deficient animals or man receiving calcium the phosphate absorption is low This is thought to be secondary to poor absorption of calcium and the consequent sequestration of calcium phosphate within the intestine [84 110]

### ABNORMALITIES IN ABSORPTION OF CALCIUM AND PHOSPHORUS IN FAMILIAL HYPOPHOSPHATEMIA AND VITAMIN D–RESISTANT RICKETS

Children with vitamin D-resistant rickets before treatment with massive doses of vitamin D excrete virtually all their ingested calcium in the feces Urinary calcium excretion is low These subjects have slightly positive or slightly negative balances for calcium but they clearly lack the positive balance which is characteristic of the normal growing child Total phosphate balance is comparable to the calcium balance but a larger proportion of the ingested phosphate is absorbed and excreted in the urine The findings are similar in the few studies of patients with vitamin D–resistant rickets with [3, 5, 17, 114] and without family histories [1 94, 115 116]

In vitamin D-resistant rickets large doses of vitamin D (2 5 to 37 5 mg per day) uniformly increase the absorption of calcium while urinary excretion remains low Simultaneously the phosphate balance becomes positive primarily because of increased absorption This response to very large doses of vitamin D is similar to the response of patients with vitamin D-deficiency rickets to smaller doses Dihydrotachysterol (A T 10) in doses in the same range as those of vitamin D (1 25 to 12 5 mg per day) produces similar changes in calcium absorption in vitamin D-resistant rickets [86 114 117]

The response of patients with resistant rickets to *massive* doses of vitamin D is similar to that of other subjects Albright and his coworkers [84–86] for example found comparable increases in calcium absorption in patients with primary hypoparathyroidism and patients with idiopathic hypercalcinuria given large doses of vitamin D or dihydrotachysterol Normal man and normal rats also have increased calcium absorption when given large doses of vitamin D [111 118]

Further evidence that poor absorption of calcium is not the only defect

in some areas Toxic doses of vitamin D clearly produce abnormal amounts of bone resorption [111] Bauer et al [11] in studies in man found that the estimated increase in total daily accretion of phosphate salt into bone due to vitamin D was two to six times the extra retention of phosphate from the gastrointestinal tract promoted by vitamin D The changes were of similar magnitude in two subjects with vitamin D-deficiency rickets treated with 30 000 to 45 000 I U per day and in two subjects with vitamin D-resistant rickets treated with 150 000 to 500 000 I U per day Thus it appears that under the influence of vitamin D calcium and phosphate are mobilized from one part of bone to be deposited in another

As Nicolaysen and Eeg Larsen have pointed out [111] it is both important and difficult in attempting to establish the actions of vitamin D to distinguish between effects of vitamin D on calcium absorption and direct effects on bone The same problem exists in the study of the pathologic physiology of vitamin D-resistant rickets One cannot say from available evidence whether the response of the bone to normal levels of vitamin D would be normal if the serum concentrations of calcium and phosphate were maintained at normal levels for prolonged periods by means other than very large doses of vitamin D

The similarity of the changes in metaphysis and diaphysis to those seen in osteochondrodystrophy raises the possibility that this bony abnormality is another congenital presumably inherited defect in these patients and not directly related to hypophosphatemia or to vitamin D The lesion could also be a direct effect of parathyroid overactivity although the changes described have not been entirely typical

## POSSIBLE MECHANISMS OF PATHOGENESIS AND QUESTIONS STILL UNANSWERED

This discussion of pathogenesis of familial hypophosphatemia and vitamin D-resistant rickets will be focused on the two abnormalities which the authors consider to be the most characteristic features of the disorder hypophosphatemia and decreased renal tubular reabsorption of phosphate The other abnormalities thus far identified—low gastrointestinal absorption of calcium and the bony abnormalities—will be discussed in so far as they bear upon the pathogenetic possibilities to be presented A final decision about mechanism cannot now be made

### *A Genetically Determined Intrinsic Renal Tubular Defect in Reabsorption of Phosphate*

This possibility offers the simplest explanation for the occurrence of hypophosphatemia and decreased renal tubular reabsorption of phosphate Consistent with this hypothesis are the facts that hypopho

changes are present in cases with and without family histories The radiologic changes in the epiphyseal area are entirely characteristic of vitamin D-deficiency rickets

A second type of abnormality has been found in the compact bone of the metaphyses of long bones In biopsies from such areas in two cases Engfeldt et al [16] observed an abnormal, irregular mosaic formation of the Haversian system and trabeculae, very little osteoid and probably an increase in osteoblastic borders and "resorption cavities Others have described similar changes [3 29] These abnormalities may be present before treatment with massive doses of vitamin D as well as afterwards [16] The histologic pattern has some features suggestive of Paget's disease or of experimental hyperparathyroidism [125] These changes are not always present In some patients bone from metaphysis has been almost normal [17, 29]

The epiphyseal lesion typical of rickets heals in most instances when the patients are treated with massive doses of vitamin D However the healing of the epiphyseal lesion is apparently not dependent upon an action of vitamin D on the bone itself but rather upon its action in promoting absorption of calcium and phosphate Healing can be initiated by supplying large amounts of phosphate intravenously or orally [119] and is accelerated by immobilization of patients at the time of osteotomy without large doses of the vitamin [21 26, 29 102] Vitamin D-resistant rickets and vitamin D-deficiency rickets are alike in not requiring vitamin D for the healing of the epiphyseal lesion Healing is also initiated in vitamin D-deficiency rickets by supplying large amounts of phosphate intravenously [119] or citric acid-sodium citrate orally [126-128] An unexplained observation which may be a valuable clue to the difference between vitamin D-deficiency rickets and vitamin D-resistant rickets is that the latter does not heal with administration of citric acid-sodium citrate [12 23 102 120 129] Further study of this difference in response to citrate is needed

The second type of abnormality of bone structure in vitamin D-resistant rickets is more difficult to interpret This distortion of trabecular structure may simply be another manifestation of rickets slow to develop and slow to heal and accordingly more evident in vitamin D-resistant rickets than in vitamin D-deficiency rickets In rats [111 130] and dogs [131] an abnormal trabecular appearance and increase in noncalcium content of the shaft of long bones is a distinctive feature of vitamin D deficiency and is not directly related to absorption of calcium and phosphate This has suggested to Nicolaysen and coworkers [111 130] and to Mellanby [131] that vitamin D has a direct function in normal bone formation Furthermore Carlsson [132] and Nicolaysen and Eeg Larsen [133] have presented evidence in vitamin D-deficient rats which suggests that vitamin D in physiologic doses causes increased bone resorption

in some areas Toxic doses of vitamin D clearly produce abnormal amounts of bone resorption [111] Bauer et al [11] in studies in man found that the estimated increase in total daily accretion of phosphate salt into bone due to vitamin D was two to six times the extra retention of phosphate from the gastrointestinal tract promoted by vitamin D The changes were of similar magnitude in two subjects with vitamin D-deficiency rickets treated with 50 000 to 45 000 I U per day and in two subjects with vitamin D-resistant rickets treated with 150 000 to 500 000 I U per day Thus it appears that under the influence of vitamin D calcium and phosphate are mobilized from one part of bone to be deposited in another

As Nicolaysen and Eeg Larsen have pointed out [111] it is both important and difficult in attempting to establish the actions of vitamin D to distinguish between effects of vitamin D on calcium absorption and direct effects on bone The same problem exists in the study of the pathologic physiology of vitamin D-resistant rickets One cannot say from available evidence whether the response of the bone to normal levels of vitamin D would be normal if the serum concentrations of calcium and phosphate were maintained at normal levels for prolonged periods by means other than very large doses of vitamin D

The similarity of the changes in metaphysis and diaphysis to those seen in osteochondrodystrophy raises the possibility that this bony abnormality is another congenital presumably inherited defect in these patients and not directly related to hypophosphatemia or to vitamin D The lesion could also be a direct effect of parathyroid overactivity although the changes described have not been entirely typical

## POSSIBLE MECHANISMS OF PATHOGENESIS AND QUESTIONS STILL UNANSWERED

This discussion of pathogenesis of familial hypophosphatemia and vitamin D-resistant rickets will be focused on the two abnormalities which the authors consider to be the most characteristic features of the disorder hypophosphatemia and decreased renal tubular reabsorption of phosphate The other abnormalities thus far identified—low gastrointestinal absorption of calcium and the bony abnormalities—will be discussed in so far as they bear upon the pathogenetic possibilities to be presented A final decision about mechanism cannot now be made

### *A Genetically Determined Intrinsic Renal Tubular Defect in Reabsorption of Phosphate*

This possibility offers the simplest explanation for the occurrence of hypophosphatemia and decreased renal tubular reabsorption of phosphate Consistent with this hypothesis are the facts that hypophos

phatemia has a simple genetic pattern, and that there is decreased tubular reabsorption of phosphate in all instances in which it has been looked for One may propose therefore *an isolated defect in the tubular mechanism* responsible for the transport of phosphate This explanation originally suggested by Robertson Harris and McCune [2] has also been offered by Dent Fanconi and others [17 27, 91, 134-136]

Certain qualifications must be made for such a proposed intrinsic tubular defect First reabsorption of phosphate is only *decreased* not absent As the data of Tables 36 2 and 36-3 show, these patients do have a significant rate of reabsorption of phosphate which is about half of normal It is possible that there may be two "sites' or mechanisms by which phosphate is reabsorbed only one of which is absent or blocked in familial hypophosphatemia

A second qualification is that the tubular phosphate-reabsorbing mechanism which is still intact in these patients is probably responsive to parathyroid hormone This is discussed below in the next section

The response of the phosphate reabsorptive mechanism in subjects with vitamin D-resistant rickets to other agents which diminish tubular reabsorption of phosphate has not been studied It is of interest that *all agents whose effect on $Tm_P$ has been quantitatively determined* (parathyroid hormone corti one glucose acetoacetate bicarbonate) lower the $Tm_P$ to approximately the range seen in familial hypophosphatemia Table 36 5 summarizes the available data It is possible that all these agents affect (perhaps indirectly) one pathway for phosphate transport while a second mechanism is independent of their effects This possibility might be studied by determining the effects of combinations of the e agents on $Tm_P$ in normal and in affected persons

Some features of this disease are not readily explicable by a simple renal tubular defect viz the decreased gastrointestinal absorption of *calcium (and phosphate) and the metaphyseal* and diaphyseal abnormalities in the bone It is possible that a (partial) defect in intestinal absorption of phosphate analogous to the renal tubular defect could be present However the patient of Albright et al [1] absorbed over 80 per cent of ingested phosphate when on a low calcium intake Gunther et al [11b] in one patient with vitamin D-resistant rickets found all but 5 per cent of ingested radiophosphorus ab orbed when it was not given with food Similar *measurements have not been reported in familial cases*

Fanconi [135] has proposed a equence of events as follows the decreased tubular reabsorption of phosphate is the primary determinant of the low serum phosphorus concentration Accordingly deposition of bone salts in osteoid tissue is subnormal Calcium absorption is in some way related to the rate of deposition of calcium in bone and is thus al o low Consistent with this proposal is the fact that healing of the bone disease and increased *calcium absorption are initiated by* large in

travenous or oral loads of phosphate [119] The last link in the above sequence, the dependence of calcium absorption upon rate of bone deposition has not been established

TABLE 36-5 EFFECTS OF VARIOUS SUBSTANCES ON MAXIMAL TUBULAR REABSORPTION OF PHOSPHATE

| Reference | Substance | Subject | $Tm_P$ before | | $Tm_P$ after | |
|---|---|---|---|---|---|---|
| | | | Mean | (Range) | Mean | (Range) |
| [ [illegible]] | Parathyroid extract intramuscularly for 3 5 days | Man | 114 | ([illegible] 148) | 68 | (31-[illegible]1) |
| [[illegible] ] | Cortisone intravenously | Dog | 140 | | 66 | |
| [[illegible]] | Cortisone orally or hydrocortisone intravenously | Man | 1[illegible]0 | (100–175) | 96 | (61–129) |
| [ 0] | Diethylstilbestrol for 14 21 days | Man | 13[illegible] | (91–194) | 100 | (67–129) |
| [74] | Acetoacetate loading | Dog | 116 | (110–1[illegible]3) | [illegible] | (64 81) |
| [ 3] | Glucose loading | Dog | 118 | (103–134) | [illegible]3 | (61–85) |
| [ ] | Glucose loading | Dog | 115 | (104–1[illegible]6) | 65 | (56–74) |
| [ 5] | NaHCO | Acidotic dog | 9 | (8 -97) | 4[illegible] | (4 50) |
| [76] | Respiratory alkalosis | Dog | 8 | (83 [illegible]3) | 6[illegible] | (64 71) |
| [5 ] | Alanine or glycine | Dog | 193 | (18[illegible] 07)† | 68§ | |
| | | | 15[illegible] | (144–15[illegible])‡ | 53§ | |

Expressed in micromoles per 100 ml glomerular filtrate

† Fasting following carbohydrate diet

‡ Fasting following meat diet

§ Based on textual statement that the $Tm_P$ fell to about 35 per cent of control value

*Hyperparathyroidism Secondary to a Defect in Gastrointestinal Absorption of Calcium*

On the basis of their studies on the first patient in whom the diagnosis of vitamin D-resistant rickets was made Albright et al [1 84] proposed that the primary event is decreased absorption of calcium because of resistance to vitamin D This leads to a low serum calcium concentration which in turn stimulates parathyroid secretion Increased parathyroid hormone activity decreases tubular reabsorption of phosphate and hypophosphatemia occurs Secondary to the hypophosphatemia (as Albright proposed) or due to another direct action of parathyroid hormone on bone (as subsequent work has shown [137–139]) resorption of calcium from bone occurs and raises the serum calcium concentration If the compensatory overactivity of the parathyroid glands should be just sufficient the final results of this sequence could be normal serum

phatemia has a simple genetic pattern and that there is decreased tubular reabsorption of phosphate in all instances in which it has been looked for One may propose therefore an isolated defect in the tubular mechanism responsible for the transport of phosphate This explanation originally suggested by Robertson Harris and McCune [2] has also been offered by Dent Fanconi and others [17, 27 91, 134–136]

Certain qualifications must be made for such a proposed intrinsic tubular defect First, reabsorption of phosphate is only *decreased* not absent As the data of Tables 36 2 and 36-3 show, these patients do have a significant rate of reabsorption of phosphate which is about half of normal It is possible that there may be two "sites' or mechanisms by which phosphate is reabsorbed, only one of which is absent or blocked in *familial hypophosphatemia*

A second qualification is that the tubular phosphate reabsorbing mechanism which is still intact in these patients is probably responsive to parathyroid hormone This is discussed below, in the next section

The response of the phosphate reabsorptive mechanism in subjects with vitamin D-resistant rickets to other agents which diminish tubular reabsorption of phosphate has not been studied It is of interest that *all agents whose effect on $Tm_P$ has been quantitatively determined* (parathyroid hormone, cortisone glucose acetoacetate bicarbonate) lower the $Tm_P$ to approximately the range seen in familial hypophosphatemia Table 36 5 summarizes the available data It is possible that all these agents affect (perhaps indirectly) one pathway for phosphate transport while a second mechanism is independent of their effects This possibility might be studied by determining the effects of combinations of these *agents on $Tm_P$ in normal and in affected persons*

Some features of this disease are not readily explicable by a simple renal tubular defect viz, the decreased gastrointestinal absorption of calcium (and phosphate) and the metaphyseal and diaphyseal abnormalities in the bone It is possible that a (partial) defect in intestinal absorption of phosphate analogous to the renal tubular defect could be present However the patient of Albright et al [1] absorbed over 80 per cent of ingested phosphate when on a low calcium intake Gunther et al [115] in one patient with vitamin D-resistant rickets found all but 5 per cent of ingested radiophosphorus absorbed when it was not given with food Similar measurements have not been reported in familial cases

Fanconi [135] has proposed a sequence of events as follows the decreased tubular reabsorption of phosphate is the primary determinant of the low serum phosphorus concentration Accordingly deposition of bone salts in osteoid tissue is subnormal Calcium absorption is in some way related to the rate of deposition of calcium in bone and is thus also low Consistent with this proposal is the fact that healing of the bone disease and increased calcium absorption are initiated by large in

Lamy et al [31] in one child with nonfamilial vitamin D-resistant rickets or a variant of the Lanconi syndrome found that near the end of a 3 hr calcium infusion the urine became phosphate free (and aminoaciduria previously present also disappeared) Fraser et al have made similar observations on phosphate excretion with more prolonged calcium infusions in two children with vitamin D-resistant rickets subcutaneous administration of parathyroid extract then restored the phosphaturia [144 145] Field and Reiss [146] found that the %TRP rose from low to normal levels in 12 affected members of three families with this disorder However since serum phosphorus concentration also remained below normal in these studies the amount of phosphorus actually being reabsorbed by the tubules remained low Only by raising serum phosphorus to normal or preferably to a level which clearly saturates tubular reabsorptive capacity, at the time of a calcium infusion could one hope to differentiate unequivocally between the role of the parathyroid glands and that of an underlying tubular defect In one such study in an affected adult member (IV 5) of the E kindred of Winters et al [8] $Tm_P$ in the sixth hour of calcium infusion (13 mg per kg over 6 hr) was low and not significantly different from two previous measurements of $Tm_P$ in the same subject (Table 36 3) Further studies of this type are needed in familial and nonfamilial instances of the disorder it is entirely possible that more than one mode of pathogenesis exists

Parathyroid extract given *intravenously* to several patients with this disorder has produced variable increases in phosphate excretion [17 28 29] It is now known as best demonstrated by Hiatt and Thompson (54) that intravenously administered parathyroid extract increases glomerular filtration rate to such a degree that no interpretation can be made about a change in tubular reabsorption

The evidence that hyperparathyroidism is *not* a prominent feature of familial hypophosphatemia is inferential In patients with hypophosphatemia only it seems improbable that a defect in calcium absorption could be so delicately balanced by parathyroid overactivity that serum calcium concentration would be maintained at a normal level throughout life without any signs of hyperparathyroidism other than increased phosphate excretion The absence of clinical or radiologic evidence of bone resorption in the asymptomatic patients is additional evidence against the presence of chronic hyperparathyroidism Histologic examination of specimens of bone would be most helpful

It is impossible to decide at present whether the hypophosphatemia and decreased renal tubular reabsorption of phosphate are due to an intrinsic renal tubular defect or to hyperparathyroidism secondary to an intestinal defect in absorption of calcium Several lines of investigation which might help to clarify this question have already been indicated It is conceivable that parathyroid hormone depresses the reabsorption

calcium (and normal ionized calcium [62]) low serum phosphorus concentration and diminished renal tubular reabsorption of phosphate which are the findings of patients with vitamin D-resistant rickets

If Albright's scheme is correct, one would expect histologic evidence of parathyroid hyperplasia and evidence of bony changes characteristic of hyperparathyroidism Albright et al [1] actually found hyperplastic changes in one parathyroid gland removed from their patient Frame and Smith [136] made a similar observation No parathyroid glands have been examined in patients with familial histories of this disease As already noted in the section on the bony abnormality, the changes that have occasionally been found in diaphyseal and metaphyseal regions have been interpreted as similar to those of experimental hyperparathyroidism Furthermore in a number of patients particularly those reported by Holt [19] the lamina dura has been absent This finding has been observed more commonly in hyperparathyroidism than in any other type of bone disease but it has not been looked for extensively in other types of rickets and osteomalacia It has been noted in certain cases of osteomalacia due to steatorrhea and to renal tubular acidosis [86]

In the single report of assay of the serum for parathyroid hormone activity in a patient who probably had resistant rickets Highman and Hamilton [140] found a higher than normal value by their calcium mobilization test Such a test is only a crude measure of a single parathyroid function and one which may not be correlated with the effects on phosphate excretion [141–143]

Renal tubular reabsorption of phosphate in vitamin D-resistant rickets is probably responsive to changes in parathyroid activity Albright et al [1] found in their patient that administration of parathyroid hormone (500 units a day intramuscularly for 3 days) caused an increase in phosphate excretion This dose and route in normal man increase phosphate excretion by decreasing tubular reabsorption without any change in glomerular filtration rate [54]

Suppression of parathyroid function has been the interpretation given to the results of intravenous calcium infusions in normal subjects Phosphate excretion usually falls following the infusion presumably because of suppression of parathyroid function by the elevated serum calcium concentration In two affected adult members of the "E kindred of Winters et al [8] intravenous calcium loading caused a 30 to 60 per cent decrease in phosphate excretion which is similar to what occurs in normal persons [63 65 66] These results suggested that the kidneys of these patients do respond to changes in parathyroid activity however in the presence of their low levels of serum phosphorus it might have been expected that the urine would have become phosphate free if increased parathyroid activity were the only factor affecting phosphate excretion

Others have observed more striking responses to calcium infusions

to normal when vitamin D is given Serum citrate concentration rises to upernormal levels when vitamin D intoxication is produced in patients with vitamin D–deficiency rickets and vitamin D–resistant rickets [39 89 90] The effects of vitamin D on citrate metabolism are apparently not necessarily related to its effects on promoting calcification of osteoid Cortisol when given to vitamin D–deficient rats prevents the rise in citrate concentration of serum and bone which vitamin D usually produces but does not block the action of vitamin D in raising the erum calcium and phosphate concentrations and healing of the rickets [150] Conversely when vitamin D-deficiency rickets in rats is healed by giving calcium and phosphate but no vitamin D the citrate content of bone and serum remains low [133] It would be interesting to know whether the healing of vitamin D–re istant rickets initiated by pho phate administration or immobilization is accompanied by changes in citrate content of bone or serum

*Other Possible Mechanisms of Pathogenesis*

Kodicek [151] in 1956 gave a preliminary report of abnormal Δ-4 3 ketosteroids in the urines of even patients with vitamin D–refractory osteomalacia unrelated to vitamin D administration Cortisone depresses tubular reabsorption of phosphate in the dog and in man under some circumstances and may decrease intestinal absorption of calcium A related steroid might well have a more marked effect on these functions and be casually related to this disease The production of such an abnormal steroid could be genetically determined

It has also been proposed that there is a generalized abnormality in the intermediary metabolism of phosphate perhaps affecting all body cells [8 28 29] Studies related to this possibility have thus far been applied to erythrocytes only [152–154] where decreased concentrations of organic phosphate compounds have been found in experimental and naturally occurring rickets It appears likely that this finding is a reflection of a generally low body content of labile phosphorus rather than the result of an abnormality in intermediary metabolism due to lack of vitamin D [155] Studies of this type have not been made in patients with familial hypophosphatemia and vitamin D–resistant rickets

An observation which may indicate a more specific abnormality in vitamin D–resistant rickets is that of Rupp [156] that erythrocytes of such a patient incorporate more radioactive phosphorus into creatine pho phate and less into adenosine triphosphate than erythrocytes of normal subjects

*Summary of Possible Mechanisms of Pathogenesis*

The development of the abnormalities in familial hypophosphatemia and vitamin D–resistant rickets is not yet understood The pre ence of a simple genetically determined renal tubular defect in reabsorption of

of phosphate of all tubules more or less equally, whereas in familial hypophosphatemia an intrinsic tubular defect might involve some tubules to a greater extent than others In the latter circumstance an increased "splay" area in the titration curve might be detectable

*An Abnormality in the Absorption of Vitamin D*

There is little direct evidence for an abnormality in the absorption of vitamin D in vitamin D-resistant rickets The blood level of the vitamin as measured by the bioassay technique of Warkany is normal prior to treatment with massive doses, and markedly elevated afterwards [?, 116 120] In addition the bone disease is not prevented or cured by *parenterally* administered conventional doses of vitamin D [1 12, 20 24 28 29] nor is ultraviolet light effective in curing rickets of the resistant type [1 10, 21 116, 116 147]

*An Abnormality in the Action of Vitamin D*

The fact that large doses of vitamin D will cure the rickets cannot be considered *prima facie* evidence that the cause of the disorder is a resistance to normal amounts of the vitamin It has already been pointed out that healing of the rachitic bone lesions is initiated by supplying adequate phosphate without additional vitamin D The action of *massive* doses of vitamin D (or comparable doses of dihydrotachysterol) on intestinal absorption of calcium is similar to that seen in persons who do not have rickets of either type

Nevertheless the possibility must be considered that these patients do have a resistance to vitamin D at the level at which it exerts its action in normal individuals This "resistance would presumably be inborn in the familial cases and acquired in the sporadic instances Support for this view comes from the descriptions of patients who have been only partially resistant and are cured by doses of intermediate size [5 148] Other patients have developed increasing resistance to vitamin D and have required larger and larger doses [149] Certain patients have seemed to respond to dihydrotachysterol when they did not respond to vitamin $D_2$ or $D_3$ in large doses [147] These last observations suggest a resistance to specific sterols as has been described for some patients with hypoparathyroidism [149] No familial case of sterol specific resistance has been described

Tobler et al [29] refer to preliminary studies by Fluckiger of a serologic reaction to vitamin D, found in the serum of patients with vitamin D-resistant rickets but not in the serum of normal persons Further evidence on this point is needed

Clues to the role of vitamin D in familial hypophosphatemia and vitamin D-resistant rickets might come from studies of citrate metabolism In vitamin D-deficiency states a low content of citrate is found in bone, intestines kidney serum and urine These levels promptly return

hypophosphatemia A clear generation to generation transmission of the trait emerged and there was no evidence of *skipping*

The same result was found not only in other portions of this same kindred but also in three additional unrelated kindreds studied subsequently [9] On the basis of these studies which include a total of 78 hypophosphatemic persons the following conclusions were drawn

1 All of the 40 persons who had skeletal disease or deformities were hypophosphatemic

2 An additional 38 individuals were hypophosphatemic but had no definite evidence past or present of skeletal disease

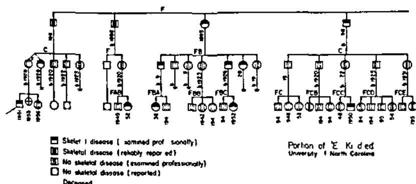



Fig 36-6 A portion of the pedigree of the F kindred of Winters et al [8] scored according to the presence of skeletal disease No clear-cut pattern of inheritance can be identified

3 *In these 38 were included all 11 instances of proved or probable* skipping which were scored when skeletal disease was used as the criterion for scoring

4 Hypophosphatemia was inherited in a clear and simple fashion each hypophosphatemic person had one hypophosphatemic parent in all of the 50 instances where the requisite data were available

5 No hypophosphatemic person had more than one hypophosphatemic parent in a number of matings where both parents were examined

6 Brothers and sisters of hypophosphatemic persons included both normal and affected persons in approximately equal numbers

On the basis of these results it is apparent that *hypophosphatemia* is inherited as a dominant trait *with a high degree of penetrance (perhaps* not complete penetrance in young girls as indicated below) but the transmission of hypophosphatemia even if fully penetrant is insufficient to produce overt bone disease There can be little doubt that hypophosphatemia is an important but not the sole determinant of bone disease

These studies also indicate that the mode of transmission is *not* autosomal but rather is sex linked This conclusion is based upon the authors

phosphate would account very well for the findings in those patients with asymptomatic hypophosphatemia but any attempt to explain the frequent additional findings—the bony abnormalities and defective absorption of calcium—on the basis of a renal tubular defect immediately becomes speculative. The possibility that the primary defect is in absorption of calcium, with an associated secondary hyperparathyroidism has been neither proved nor disproved but seems an unlikely explanation for the existence of asymptomatic hypophosphatemic individuals in the involved families. There is no direct supporting evidence for the proposal that there is an abnormality in the metabolism of vitamin D or a resistance to its action at normal levels. The action of large doses in curing the rachitic lesions can be explained in terms of the action of such doses in normal persons. The evidence for a disturbance in steroid metabolism may be a promising lead.

## GENETIC ASPECTS

There is a definite tendency for vitamin D-resistant rickets to occur in families. In 65 adequately documented cases in the literature prior to 1958 over half had a positive family history [8]. From studies of small pedigrees in which affected individuals were scored on the basis of overt bone disease, several workers [5, 6] advanced the hypothesis that the disease was transmitted as an autosomal dominant trait. However, the overt form of the disease had been reported more frequently in females than in males. Analysis of the distribution of normal and affected females and males among the progeny of affected persons in the earlier studies revealed a deviation from the 1:1:1:1 ratio predicted by the autosomal dominant hypothesis. The primary cause of this deviation was an excess of affected females [8]. These considerations suggested either that there is a strong influence of sex on the expression of an autosomal trait or that some other mode of transmission is operative.

Winters et al. [8] reinvestigated the genetic aspects of the disease in a large North Carolina kindred ("E" kindred). Individuals were scored not only on the basis of skeletal disease but also on the basis of the level of serum inorganic phosphorus (see Hypophosphatemia and the Abnormality in Renal Excretion of Phosphate). Comparison of these two methods of scoring revealed that the latter is a superior discriminant for the trait. This point is illustrated in Figs. 36-6 and 36-7 where a portion of the "E" kindred is reproduced. In Fig. 36-6 individuals were scored as affected on the basis of clinical, radiographic, or historical evidence of active rickets (children) or postrachitic deformities (adults). Using this method, there was no clear-cut pattern of inheritance and several instances of "skipping" of a generation were noted. This may be contrasted with the findings in Fig. 36-7 where scoring was based upon

own studies of four kindreds In the *classified* progeny of affected fathers all 16 males were normal while all 20 of the females were affected (Table 36 6) The chance of this distribution occurring on the basis of an autosomal dominant mechanism is virtually nil

A sex linked dominant mechanism implies that the abnormal gene resides on the X chromosome An affected female is therefore heterozygous (X*X) whereas an affected male lacking the normal allele is hemizygous (X*Y) Since the male progeny of an affected male receive their Y chromosome from their fathers they would all be normal On the other hand female progeny of an affected male receive the single X* chromosome of the father and are therefore all affected The sex linked dominant hypothesis also predicts that half the progeny of an affected

TABLE 36-6 DATA ON FOUR KINDREDS OF HYPOPHOSPHATEMIC PARENTS

| *Sex of progeny* | *Affected* | *Normal* | *Total* |
|---|---|---|---|
| Classified progeny of hypophosphatemic fathers | | | |
| Male | 0 | 16 | 16 |
| Female | 20 | 0 | 20 |
| Total | 0 | 16 | 36 |
| Classified progeny of hypophosphatemic mothers | | | |
| Male | 20 | 13 | 33 |
| Female | 10 | 24 | 34 |
| Total | 30 | 37 | 67 |

SOURCE R W Winters et al [8] and J B Graham et al [9]

female should be normal and half affected without regard for sex Data on this point are also shown in Table 36-6 and demonstrate a significant deviation from the expected distribution due primarily to an excess of normal daughter Although the complete explanation for this unexpected distribution is not clear it is noteworthy that many of the daughters scored as normal were in the first decade where the discriminant for the diagnosis of hypophosphatemia gives a larger rate of false negative diagnoses than at any other age group in either sex [9 30]

The sex linked dominant hypothe is not only explains more adequately than the autosomal dominant mechani m the results of the authors own studies but also offers a better explanation for the previously reported genetic data No pedigree published by other workers is sufficiently large to reject the autosomal hypothesis with confidence but all are equally well explained by the sex linked dominant mechanism The latter hypothesis also accounts for the excess of affected females in families previously described The clearest rejection of the sex linkage would be a documented instance of male to male transmission Although there is a

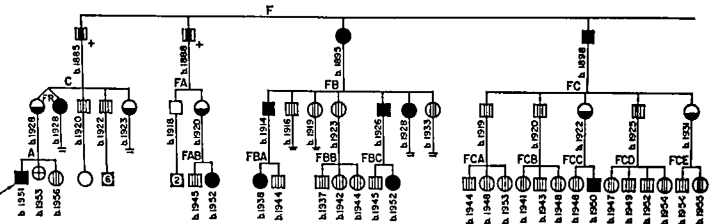



Fig 36-7 The same pedigree as that shown in Fig 36-6 scored according to the presence of hypophosphatemia Generation-to-generation transmission with no evidence of "skipping" can now be seen

variety A recently studied sporadic case [107] was seen in a severely affected *female* who showed no clinical or chemical features distinguishing her from patients with the sex linked variety of the disease Both parents and many relatives of this patient had no clinically or chemically identifiable related disease The disease in this and similar patients could be the result of a new mutation or of a recessive trait or could represent a phenocopy

## TREATMENT

The treatment of vitamin D resistant rickets is highly individualized it must be based on recognition not only of the age and growth potential of the patient and the severity of his skeletal disease but also of the very real risks of hypervitaminosis D

### *Vitamin D Therapy*

It is in children with overt bone disease that the need for therapy is most pressing The general plan of therapy is to start with a dose of vitamin D[1] of 25 000 to 50 000 I U daily This is then increased by increments of 25 000 I U per day at intervals of 1 month to 6 weeks Increases are determined by chemical evidence (principally a falling level of alkaline phosphatase in the serum) or radiographic evidence of healing The dosage necessary to effect clear cut healing is variable but is generally of the order of 150 000 to 250 000 I U per day Once healing has been initiated there is no assurance that the dose may remain constant subsequent alterations are often necessary in order to avoid reactivation on the one hand or cumulative vitamin D poisoning on the other

The risks of hypervitaminosis D are considerable In some patients the effective therapeutic dose lies in the frankly toxic range In order to avoid serious degrees of hypercalcemia continuous medical supervision is mandatory and should include a careful search for signs and symptoms of hypercalcemia serial measurements of serum calcium and frequent urinalyses and estimations of blood urea nitrogen Mild but significant hypercalcemia is often unaccompanied by objective signs and clear cut symptoms The Sulkowitch test for calcium in the urine is not of great value in the early recognition of vitamin D poisoning but it may be

[1] Vitamin D (calciferol) is most widely used Vitamin D is also effective as is dihydrotachysterol (A T 10) but the latter two agents are not preferred because of expense and relative unavailability The fact that dihydrotachysterol in doses which increase the intestinal absorption of calcium [8 11] will heal vitamin D-resistant rickets whereas its effectiveness in healing ordinary rickets is poor compared with vitamin D is further evidence for the view that the corrective action of these sterols in vitamin D-resistant rickets is a nonspecific effect on calcium absorption Consistent with this view is the healing action of dihydrotachysterol in vitamin D-*deficiency* rickets due to a low-calcium diet in rats [158]

single report suggesting this occurrence [10] the mother of the affected boy was not examined for hypophosphatemia

In the first kindred studied by Winters et al [7, 8] a marked difference was observed between *heterozygotes and hemizygotes with respect to* the severity of bone disease Hypophosphatemic males nearly always had obvious rickets or postrachitic deformities while hypophosphatemic females tended to have minimal skeletal involvement In general this difference has been confirmed in the three pedigrees subsequently studied [9] A summary of these findings appears in Table 36 7 Although exact *rating of severity of bone disease is admittedly difficult, there can be* little doubt about the tendency of males to be more severely affected than females

TABLE 36-7 CLASSIFICATION OF HYPOPHOSPHATEMIC PERSONS BY SEXES ACCORDING TO SEVERITY OF BONE DISEASE

| | *Absent* | *Mild* | *Moderate or severe* | *Total* |
|---|---|---|---|---|
| Males | 2 | 1 | 27 | 30 |
| Females | 36 | 2 | 10 | 48 |
| Total | 38 | 3 | 37 | 78 |

SOURCE From studies of four kindreds by R W Winters et al [8] and J B Graham et al [9] Classification made according to the general criteria used in [8]

In searching for an explanation for these differences statistical comparison of the levels of serum phosphorus was made between the various subgroups of heterozygotes and hemizygotes in the E kindred In order to make any valid comparison of this type the data were age adjusted Males in various age groups had lower levels of serum phosphorus than females in the corresponding groups These differences although statistically significant were small The largest was 0 47 mg per 100 ml between affected male and female children In view of the *uncertainties about the method of age adjustment* the significance of these differences must remain uncertain

The relative immunity of the female from severe skeletal disease has at least two possible explanations (1) the normal *allele may* modify the action of the abnormal gene so that the female escapes severe bone disease because of heterozygosity (2) the relative immunity of the female is a factor operative in that sex independent of gene dosage Since there is no proved instance of a homozygous female this problem remains unresolved

Some previous authors have reported cases of resistant rickets in which both parents were clinically normal (cf Winters et al [8] for review) *In no instance have the parents been examined chemically*

Not all cases of vitamin D–resistant rickets are of the sex linked

The treatment of osteomalacia should proceed along the lines outlined above for the treatment of affected children

In affected adults without osteomalacia the problems are those of residual deformities and the secondary arthritic complications Treatment with vitamin D is not indicated for these problems

*Treatment of Asymptomatic Hypophosphatemia*

There would seem to be little reason to treat patients with asymptomatic hypophosphatemia If it could be shown that treatment with vitamin D improved the growth of such children then therapy might be considered

## PREVENTIVE MEASURES

Once the mode of inheritance of the trait has been determined in a particular family the affected individuals and their spouses should be fully informed of the nature of the transmission of the gene as well as the chances and probable consequences of having a child with the disease Potentially affected progeny of such a mating should then be studied as early as practicable in order to establish the genotype on the reasonable assumption that institution of therapy may prevent serious degrees of deformity As noted earlier (see Hypophosphatemia above) the detection of hypophosphatemia is difficult in a young infant The diagnosis can be made or excluded with confidence only by obtaining multiple determinations

## SUMMARY

1 Familial hypophosphatemia and vitamin D-resistant rickets is a disorder in which the most distinctive features are hypophosphatemia and diminished renal tubular reabsorption of inorganic phosphate

2 The inheritance pattern is that of sex linked dominance with complete penetrance

3 The rickets or osteomalacia which may develop is not responsive to usual therapeutic doses of vitamin D but can be healed with massive doses of the vitamin

4 Children with bony involvement probably have abnormally low absorption of calcium from the gastrointestinal tract This responds only to large amounts of vitamin D a response similar to the effects of large doses of vitamin D on intestinal absorption of calcium in normal individuals

5 Healing of the rickets can be initiated by measures which do not involve vitamin D such as immobilization or administration of large amounts of phosphate It thus appears that the bony involvement is secondary to the disorder in phosphate metabolism rather than to a lack of a direct action of vitamin D on bone

helpful to have the parents perform the test regularly to focus their attention upon this ever present problem

In most instances careful adjustment of the dosage of vitamin D is successful in controlling the active rachitic process As healing occurs the level of alkaline phosphatase in the serum falls to normal Phosphorus balance becomes positive but serum phosphorus does not often rise to clearly normal values At first glance this may seem to contradict the hypothesis that hypophosphatemia is a dominant determinant of bone disease However the experiments of Yendt and Howard [159] provide an explanation They found that transient increases in the level of phosphate in rachitic rats may be sufficient to initiate calcification, which continues even though the ambient concentration of phosphate is again reduced to levels associated with rickets

Healing of the rickets is often associated with some improvement in the severity of the deformities although in cases with serious degrees of deformity subsequent osteotomy may be indicated Surgical correction should be undertaken only after the active process is fully controlled Before and during the period of immobilization the dosage of vitamin D must be greatly reduced or eliminated and then reinstituted with mobilization

Another major unsolved problem in children is that although the rachitic process may be optimally controlled by vitamin D, the expected increase in the rate of linear growth does not often occur and the patients remain dwarfed The possibility that continuing hypophosphatemia limits linear growth independently of its effect upon the rachitic lesion deserves further study

### *Other Therapeutic Measures*

It has been shown [114] that adding inorganic phosphate to vitamin D therapy may increase phosphate and calcium retention restoring the serum phosphorus concentration to normal The administration of phosphate will initiate healing even in the absence of vitamin D [119] However after more extended observations Fraser has concluded that long term phosphate therapy has no proved place in the management of vitamin D–resistant rickets and may even be harmful [95]

### *Treatment of Affected Adults*

The rarity of overt osteomalacia in adults with hypophosphatemia and a history of vitamin D–resistant rickets is remarkable In the large kindred studied by Winters et al [8] there were 23 adequately studied hypophosphatemic adults Only 2 of these had evidence of osteomalacia in the form of single pseudofractures A few affected adults with serious degrees of osteomalacia have been reported by others [5 28, 91 92 101]

genetic study of primary rickets with familial disposition Acta paediat (Uppsala) 47 ?88 1958
14 Coleman E N and Foote J B Craniostenosis with familial vitamin D resistant rickets Brit M J 1 561 1954
15 Daeschner C W Jr Vitamin D resistant rickets diagnosis and management Texas J Med 53 324 1957
16 Engfeldt B Fetterstrom R and Winberg J Primary vitamin D resistant rickets III Biophysical studies of skeletal tissue J Bone & Joint Surg 38 A 1323 1956
17 Fanconi G and Girardet 1 Familiärer persistierender Phosphatdiabetes mit D vitamin resistenter Rachitis Helvet paediat acta 7 14 1952
18 Gardner L I Vitamin D refractory rickets in Textbook of Pediatrics edited by W E Nelson 6th ed Saunders Philadelphia 1954
19 Holt J F Vitamin D resistant rickets (refractory rickets) Am J Roentgenol 64 590 1950
20 Imerslund O Craniostenosis and vitamin D resistant rickets Acta paediat (Uppsala) 40 449 1951
21 Lamy M Royer P Frézal J and Lestradet H Le Rachitisme vitaminorésistant familial hypophosphatémique primitif Arch franç pédiat 15 1 1958
22 Linnerloth K Hallgren B Ialmén K and Zetterström R Primary (genuine) vitamin D resistant rickets IV A clinical and genetic study Acta paediat (Uppsala) 47 568 1958
23 Litman N N Ulstrom R A and Westin W W Vitamin D resistant rickets California Med 86 ?48 1957
24 MacKay H and May Q I Rickets resistant to vitamin D healing with very heavy dosage of vitamin D fluctuations in vitamin D requirement development of hypercalcemia Proc Roy Soc Med 38 565 1944–1945
25 McCune D J Refractory rickets in identical twins Am J Dis Child 63 1008 1942
26 Pedersen H E and McCarroll H R Vitamin resistant rickets J Bone & Joint Surg 33 A 203 1951
27 Peterson R E Hypophosphatemic rickets Description and case reports of renal tubular form of this deficiency disease J Kansas M Soc 57 58 1956
28 Swoboda W Die genuine Vitamin D-resistente Rachitis Wien Beitr Kinderh 6 1 1956
29 Tobler R Prader A and Taillard W Die familiare primäre vitamin D resistent Rachitis (Phosphatdiabetes) Helvet paediat acta 11 ?03 1956
29a Hsia D Y Y Kraus M and Samuels J Genetic studies on vitamin D resistant rickets (familial hypophosphatemia) Am J Human Genet 11 156 1959
30 Greenberg B G Winters R W and Graham J B The normal range of serum inorganic phosphorus and its utility as a discriminant in the diagnosis of congenital hypophosphatemia J Clin Endocrinol 20 364 1960
31 Hopkins T Howard J L and Lisenberg H Ultrafiltration studies on calcium and phosphorus in human serum Bull Johns Hopkins Hosp 91 1 1952
32 Hogben C A M and Bollman J L Renal reabsorption of phosphate normal and thyroparathyroidectomized dog Am J Physiol 164 6 0 1951
33 Handler P and Cohn D V Use of radiophosphorus in studies of glomerular permeability of plasma inorganic phosphate Am J Physiol 164 646 1951
34 White H L Further observations on glomerular function Am J Physiol 102 2?2 1932
35 Walker A M and Hudson C L The role of the tubule in the excretion of inorganic phosphate by the amphibian kidney Am J Physiol 118 16 193
36 Pitts R F Gurd R S Kessler R H and Hierholzer K Localization of acidification of urine potassium and ammonia secretion and phosphate reabsorption in the nephron of the dog Am J Physiol 194 125 1958
37 Barclay J A Cooke W T and Kenney R A Evidence for a three-component system of renal excretion Acta med scandinav 128 500 194

6 One of the leading explanations proposed for the disease is that there is an intrinsic defect in the function of the renal tubular cells If so the defect is highly specific for it is not associated with any other known tubular defect Furthermore the aminoaciduria which accompanies vitamin D-deficiency rickets *is not found in individuals with familial* hypophosphatemia

7 The second leading explanation is that there is overactivity of the parathyroids, secondary to diminished intestinal absorption of calcium *which depresses renal tubular reabsorption of pho phate* If so, the compensatory adjustment is remarkably exact for serum calcium concentration is almost always normal and hyperparathyroidism is not detectable clinically

8 Studies of the quantitative aspects of tubular reabsorption of phosphate in relation to parathyroid function to the action of vitamin D and to the reabsorption of other substances which may affect phosphate reabsorption are needed to clarify the fundamental abnormality in familial hypophosphatemia

9 From genetic considerations it is possible to predict individuals likely to develop the disorder, and to take the necessary steps toward early detection and prevention of the deforming features of this disease

## BIBLIOGRAPHY


1 Albright F Butler A M and Bloomberg E Rickets resistant to vitamin D therapy Am J Dis Child 54 5 J 1937

2 Robertson B R Harris R C and McCune D J Refractory rickets mechanism of therapeutic action of calciferol Am J Dis Child 64 918 1942

3 Christensen J F Three familial cases of atypical late rickets Acta paediat (Upp ala) 28 247 1940-1941

4 Baagøe K Rachitis tarda Ugesk laeger 86 175 1924

5 Dent C E and Harris H Hereditary forms of rickets and osteomalacia J Bone & Joint Surg 38 B 204 1956

6 Mitchell F N and Mitchell J E Vitamin D resistant rickets A M A J Dis Child 93 385 1957

7 Winters R W Graham J B Williams T F McFalls V W and Burnett C H A genetic study of familial hypophosphatemia and vitamin D resistant rickets Tr A Am Physicians 70 234 1957

8 Winters R W Graham J B Williams T F McFalls V W and Burnett C H A genetic study of familial hypophosphatemia and vitamin D resistant *rickets with a review of the literature* Medicine 37 97 1958

9 Graham J B McFalls V W and Winters R W Familial hypophosphatemia with vitamin D resistant rickets II Three additional kindred of the sex linked dominant type with a genetic analysis of four such familie Am J Human Genet 11 311 1959

10 Liebe S Über nicht heilbare Rachitis Monatsschr Kinderh 78 ? 1 1939

11 Bauer G O H Carlsson A and Lundquist B Bone salt metabolism in human rickets studied with radioactive phosphorus Metabolism 5 573 1956

12 Carlgren L E A case of vitamin D resistant rickets treated with massive doses of vitamin $D_2$ Acta paediat (Uppsala) 35 367 1948

13 Christiansson G Emergence of primary vitamin D resistant rickets at puberty

61 Albright F and Reifenstein F C Jr *The Parathyroid Glands and Metabolic Bone Disease Selected Studies* Williams & Wilkins Baltimore 1948
6 Fanconi A and Rose G A The ionized complexed and protein bound fractions of calcium in plasma Quart J Med N S 27 463 1958
63 Howard J E Hopkins T R and Connor T B On certain physiologic responses to intravenous injection of calcium salts into normal hyperparathyroid and hypoparathyroid persons J Clin Endocrinol 13 1 1953
64 Baylor C H Van Alstine H E Keutmann E H and Bassett S H The fate of intravenously administered calcium Effect on urinary calcium and phosphorus fecal calcium and calcium phosphorus balance J Clin Invest 29 1167 1950
65 Hiatt H H and Thompson D D Some effects of intravenously administered calcium on inorganic phosphate metabolism J Clin Invest 36 573 1957
66 Chambers E L Jr Gordon G S Goldman L and Reifenstein E C Jr Tests for hyperparathyroidism tubular reabsorption of phosphate phosphate deprivation and calcium infusion J Clin Endocrinol 16 1507 1956
67 Roberts K E and Pitts R F The effects of cortisone and desoxycorticosterone on the renal tubular reabsorption of phosphate and the excretion of titratable acid and potassium in dogs Endocrinology 52 3_4 1953
68 Roberts K E and Randall H T The effect of adrenal steroids on renal mechanisms of electrolyte excretion Ann New York Acad Sc 61 306 1955
69 Mills J N and Thomas S The acute effects of cortisone and cortisol upon renal function in man J Endocrinol 17 41 1958
70 Nassim J R Saville P D and Mulligan L The effect of stilboestrol on urinary phosphate excretion Clin Sc 15 367 1956
71 Beisel W B Zerzan C J Jr Rubini M E and Blythe W B Phosphaturesis a direct renal effect of triiodothyronine Am J Physiol 195 357 1958
7 Pitts R F and Alexander R S The renal reabsorptive mechanism for inorganic phosphate in normal and acidotic dogs Am J Physiol 142 648 1944
73 Cohen J J Berglund F and Lotspeich W D Renal tubular reabsorption of acetoacetate inorganic sulfate and inorganic phosphate in the dog as affected by glucose and phlorizin Am J Physiol 184 91 1956
74 Cohen J J Berglund F and Lotspeich W D Interrelations during renal tubular reabsorption in the dog among several anions showing a sensitivity to glucose and phlorizin Am J Physiol 189 331 1957
75 Malvin R L and Lotspeich W D Relation between tubular transport of inorganic phosphate and bicarbonate in the dog Am J Physiol 187 51 1956
76 Berliner R W Kennedy T J Jr and Orloff J Relationship between acidification of the urine and potassium metabolism Effect of carbonic anhydrase inhibition on potassium excretion Am J Med 11 274 1951
76a Lotspeich W D *Metabolic Aspects of Renal Function* p 15 ff Charles C Thomas Springfield Ill 1959
77 Mahler R F and Stanbury S W Potassium losing renal disease Renal and metabolic observations on a patient sustaining renal wasting of potassium Quart J Med N S 25 21 1956
78 Schiess W A Ayer J L Lotspeich W D and Pitts R F The renal regulation of acid base balance in man II Factors affecting the excretion of titratable acid by the normal human subject J Clin Invest 27 57 1948
79 Harrison H E and Harrison H C The effect of acidosis upon the renal tubular reabsorption of phosphate Am J Physiol 134 781 1941
80 Nordin B E C and Fraser R The indirect assessment of parathyroid function in *Bone Structure and Metabolism* Ciba Foundation Symposium edited by G E W Wolstenholme and C M O Connor p 2 ff Little Brown Boston 1956
81 Stalder G Endogene Phosphat-clearance bei Vitamin D Mangel rachitis und rachitogener Tetanie ihre Beeinflussung durch Vitamin D Internat Ztschr Vitaminforsch 27 382 1956 19 7
8 Harrison H E and Harrison H C The renal excretion of inorganic phosphate

38 Cooke W T Barclay J A Cowan A D T, and Nagley L Osteoporosis associated with low serum phosphorus and renal glycosuria Arch Int Med 80 117 1947
39 Harrison H E Mechanisms of action of vitamin D Pediatrics 14 285 1954
40 Ingbar S H Kass E H Burnett C H Relman A S Burrows B A and Sisson J H The effects of ACTH and cortisone on the renal tubular transport of uric acid phosphorus and electrolytes in patients with normal renal and adrenal function J Lab & Clin Med 38 533 1951
41 Levinsky N G and Davidson D G Renal action of parathyroid extract in the chicken Am J Physiol 191 530 1957
42 Laugner R Bubnoff M V and Braun W Gibt es eine tubuläre Phosphatsekretion? Über die Ausscheidung von anorganischen und organischen Phosphat bei der Katze Arch ges Physiol 258 133 1953 1954
42a Handler J and Wojtczak A Personal communication
43 Anderson J A method for estimating Tm for phosphate in man J Physiol (London) 130 268 1955
44 Bartter F C The parathyroids Ann Rev Physiol 16 429 1954
45 Longson D Mills J N Thomas S and Yates P A Handling of phosphate by the human kidney at high plasma concentrations J Physiol (London) 131 555 1956
46 Lambert P P Van Kessel E and Leplat Ch Étude sur l'élimination des phosphates inorganiques chez l'homme Acta med scandinav 128 386 1947
47 Stalder G Schmidt R and Gerstner F Die maximal tubuläre Phosphatrückresorption (Tmp) in den Nieren des gesunden Kindes Ann paediat 189 213 1957
48 Thompson D D and Hiatt H H Renal reabsorption of phosphate in normal *human subjects and in patients with parathyroid disease* J Clin Invest 36 550 1957
49 Williams T F DeWalt J L Winters R W Hollander W Jr Welt L G and Burnett C H Comparison of methods for evaluating renal excretion of phosphate in hyperparathyroidism Clin Res 6 248 1958
50 Milne M D Stanbury S W and Thomson A E Observations on the Fanconi syndrome and renal hyperchloraemic acidosis in the adult Quart J Med N S 21 61 1952
51 Goldman R and Bassett S H Phosphorus excretion in renal failure J clin Invest 33 1623 1954
52 Ayer J L Schiess W A and Pitts R F Independence of phosphate reabsorption and glomerular filtration in the dog Am J Physiol 151 168 1947
53 Sirota J H Renal tubular reabsorption of phosphate in hyperparathyroidism before and after removal of parathyroid adenoma Fed Proc 12 133 1953
54 Hiatt H H and Thompson D D The effects of parathyroid extract on renal function in man J Clin Invest 36 557 1957
55 Rupp W and Swoboda W Untersuchungen des P-O Stoffwechsels bei vitamin D resistenter Rachitis (Phosphatdiabetes) III Mitteilung Ergebnisse bei Über und Unterfunktion der Nebenschilddrüsen Helvet paediat acta 11, 256 1956
56 Lotspeich W D Kidney water and electrolyte metabolism Ann Rev Physiol 20 339 1958
57 Crawford J D Osborne M M Jr Talbot N B Terry M L and Morrill M F The parathyroid glands and phosphorus homeostasis J Clin Invest 29 1448 1950
58 Thompson D D and Hiatt H H Effects of phosphate loading and depletion on the renal excretion and reabsorption of inorganic phosphate J Clin Invest 36 566 1957
59 Stoerk H C and Carnes W H The relationship of the dietary Ca P ratio to serum Ca and to parathyroid volume J Nutrition 29 43 1945
60 Sinclair J G Fetal rat parathyroids as affected by changes in maternal serum calcium and phosphorus through parathyroidectomy and dietary control J Nutrition 23 141 1942 see also earlier references contained in [61] p 47

in relation to the action of vitamin D and parathyroid hormone J Clin Invest 20 47 1941
83 Harrison H L Mechanisms of action of vitamin D Pediatrics 14 285 1954
84 Albright F and Sulkowitch H W The effect of vitamin D on calcium and phosphorus metabolism studies on four patients J Clin Invest 17 305 1938
85 Albright F Bloomberg E Drake T and Sulkowitch H W A comparison of the effects of A T 10 (dihydrotachysterol) and vitamin D on calcium and phosphorus metabolism in hypoparathyroidism J Clin Invest 17, 317 1938
86 Albright F Burnett C H Parson W Reifenstein E C Jr and Roos A Osteomalacia and late rickets The various etiologies met in the United States with emphasis on that resulting from a specific form of renal acidosis the therapeutic indications for each etiological sub-group and the relationship between osteomalacia and Milkman s syndrome Medicine 25 399 1946
87 Rupp W and Swoboda W Untersuchungen des $PO_4$ stoffwechsels bei vitamin D-resistenter Rachitis ( Phosphat Diabetes ) I Mitteilung Helvet paediat acta 9 249 1954 II Mitteilung Helvet paediat acta 10 135 1955
88 Loudon M and Zweymuller E Beitrag zur experimentellen Vitamin D-Vergiftung beim Hund III Mitteilung Die Wirkung fraktionerter Vitamin $D_2$-Gaben auf die Nierenfunktion Ztschr ges exper Med 129 375 1955
89 Harrison H E and Harrison H C Vitamin D and citrate metabolism studies on rachitic infants Yale J Biol & Med 24 273 1951-1952
90 Bellin S A and Steenbock H Vitamin D and citraturia J Biol Chem 194, 311 1952
91 Dent C E Rickets and osteomalacia from renal tubular defects J Bone & Joint Surg 34 B 266 1952
92 McCance R A Osteomalacia with Looser s nodes (Milkman s syndrome) due to a raised resistance to vitamin D acquired about the age of 15 years Quart J Med N S 16 33 1947
93 Rupp W and Swoboda W Clearance Untersuchungen bei Vitamin D resistenter Rachitis Monatsschr Kinderh 102 173 1954
94 Winberg J Bergstrand C G Engfeldt B and Zetterstrom R Primary vitamin D refractory rickets I Report of two cases treated with high doses of vitamin D Acta paediat (Uppsala) 43 347 1954
95 Fraser D Personal communication
96 Bickel H Discussion of [97] Helvet paediat acta 10 257 1955
97 Jonxis J H P Amino aciduria and rickets Helvet paediat acta 10 245 1955
98 Jonxis J H P Smith P A and Huisman T H J Rickets and amino aciduria Lancet 2 1015 1953
99 Fishman W H Methionine induced amino aciduria in vitamin D resistant rickets Metabolism 4 107 1955
100 Freeman S and Dunsky I Resistant rickets Am J Dis Child 79 409 1950
101 Milkman L A Multiple spontaneous idiopathic symmetrical fractures Am J Roentgenol 32 622 1934
102 Kajdi L Comparison of the effect of vitamin D and citrates on mineral metabolism in late rickets Am J Dis Child 68 352 1944
103 Robinson H W and Nelson W E Phosphorus clearance in children with vitamin D resistant rickets Am J Dis Child 69 323 1945
104 Klein R and Gow R C Interaction of parathyroid hormone and vitamin D on the renal excretion of phosphate J Clin Endocrinol 13 271 1953
105 Dent C E Anderson J and Senior B Effect of vitamin D on renal reabsorption of phosphorus (Abstract The Bone and Tooth Society ) J Bone & Joint Surg 37 B 171 1955
106 Zetterstrom R and Winberg J Primary vitamin D refractory rickets II Metabolic studies during treatment with massive doses of vitamin D Acta paediat (Uppsala) 44 45 1955
107 Bauer W Albright F and Aub J C Studies of calcium and phosphorus metabolism II The calcium excretion of normal individuals on a low calcium diet also data on a case of pregnancy J Clin Invest 7 75 1929

108 Sherman H C and Hawley L Calcium and phosphorus metabolism in child hood J Biol Chem 53 3,5 192?
109 Stearns G Oelke M J and Boyd J D Mineral metabolism in late rickets Am J Dis Child 42 88 1931
110 Nicolaysen R XV Studies upon the mode of action of vitamin D III The influence of vitamin D on the absorption of calcium and phosphorus in the rat Biochem J 31 12? 1937
111 Nicolaysen R and Eeg Larsen N The biochemistry and physiology of vitamin D Vitamins & Hormones 11 ?9 1953
112 Orr W J Holt L E Jr Wilkins L and Boone F H The calcium and phosphorus metabolism in rickets with special reference to ultraviolet ray therapy Am J Dis Child 26 36? 19 3
113 Telfer S V Studies in calcium and phosphorus metabolism Part V Infantile rickets The excretion and absorption of the mineral elements and the influence of fats in the diet on mineral absorption Quart J Med 20 ? 19 6–19 7
114 Saville P D Nassim J R Stevenson F H Mulligan L and Carey M The effect of A T 10 on calcium and phosphorus metabolism in resistant rickets Clin Sc 14 489 1955
115 Gunther L Cohn E T Cohn W E and Greenberg D M Metabolism of bone salts in resistant rickets Report of a case with balance and radioactive tracer studies Am J Dis Child 66 517 1943
116 Bakwin H Bodansky O and Schorr R Refractory rickets Am J Dis Child 59 560 1940
117 Albright F Sulkowitch H W and Bloomberg E A comparison of the effects of vitamin D dihydrotachysterol (A T 10) and parathyroid extract on the disordered metabolism of rickets J Clin Invest 18 165 1939
118 Bauer W Marble A and Claflin D Studies on the mode of action of irradiated ergosterol I Its effect on the calcium phosphorus and nitrogen metabolism of normal individuals J Clin Invest 11 1 1932
119 Fraser D Geiger D W Munn J D Slater P E Jahn R and Liu E Calcification studies in clinical vitamin D deficiency and in hypophosphatemic vitamin D refractory rickets the induction of calcium deposition in rachitic cartilage without the administration of vitamin D A M A J Dis Child 96 460 1958
1 0 Eliot M M and Park E A Rickets in *Brennemann s Practice of Pediatrics* edited by I McQuarrie vol I chap 36 W F Prior Co Hagerstown 1938
121 Neuman W F and Neuman M W *The Chemical Dynamics of Bone Mineral* University of Chicago Press Chicago 1958
1 Bourne G H Ed *The Biochemistry and Physiology of Bone* Academic Press Inc New York 1956
123 Gregersen E Primary vitamin resistant rickets Acta paediat (Uppsala) 44 491 1955
124 Lussy M Über sogenannte vitamin resistente Rachitis Ann paed at 166 11 1946
1 5 Engfeldt B and Zetterstrom R Biophysical and chemical investigation on bone tissue in experimental hyperparathyroidism Endocrinology 54 50? 1954
1 6 Shohl A T and Butler A M Citrates in the treatment of infantile rickets New England J Med 220 515 1939
1?7 Shohl A T The effect of the acid base content of the diet upon the production and cure of rickets with special reference to citrates J Nutrition 14 69 1937
1?8 Hamilton B and Dewar M M Effect of citrate and tartrate on experimental rickets Am J Dis Child 54 548 1937
1 9 Fraser D Discussion of [117] A M A J Dis Ch ld 96 463 1958
130 Nicolaysen R and Jansen J Vitamin D and bone formation in rats Acta paediat (Uppsala) 23 405 1939
131 Mellanby E The rickets producing and anti-calcifying action of phytate J Physiol (London) 109 488 1949
132 Carlsson A Tracer experiments on the effect of vitamin D on the skeletal metabolism of calcium and phosphorus Acta physiol scandinav 26 21? 1952

133 Nicolaysen R and Eeg Larsen N The mode of action of vitamin D in *Bone Structure and Metabolism* Ciba Foundation Symposium edited by G E W Wolstenholme and C M O Connor p 175 ff Little Brown Boston 1956
134 Fanconi G Tubular insufficiency and renal dwarfism Arch Dis Childhood 29 1 1954
135 Fanconi G Variations in sensitivity to vitamin D from vitamin D resistant rickets vitamin D avitaminotic rickets and hypervitaminosis D to idiopathic hypercalcemia in *Bone Structure and Metabolism* Ciba Foundation Symposium edited by G E W Wolstenholme and C M O Connor p 187 ff Little Brown Boston 1956
136 Frame B and Smith R W Jr Phosphate diabetes Am J Med 25 771 1958
137 Barnicot N A The local action of the parathyroid and other tissues on bone in intracerebral grafts J Anat 82 233 1948
138 Chang H Grafts of parathyroid and other tissues to bone Anat Rec 111 23 1951
139 Stewart G S and Bowen H F The parathyroid control of serum calcium independent of renal mediation Endocrinology 48 568 1951
140 Highman W J and Hamilton B Calcium and phosphorus metabolism in a case of intractable rickets J Pediat 9 56 1936
141 Kenny A D Vine B G and Munson P L Estimation of ratio of phosphaturic and calcium mobilizing activities in parathyroid extracts Fed Proc 13 241 1954
142 Davies B M A Gordon A H and Mussett M V A mouse urine phosphate assay for parathyroid hormone with certain applications J Physiol (London) 130 79 1955
143 L Heureux M V and Melius P Differential centrifugation of bovine parathyroid tissue Biochim et biophys acta 20 447 1956
144 Fraser D Leeming J Cerwenka E A and Kenyeres K The role of parathyroid hormone in the pathogenesis of hypophosphatemic vitamin D-refractory rickets of the simple form Canadian Society for Clinical Investigation (abstract) January 1959 (Personal communication )
145 Fraser D Leeming J M Cerwenka E A and Kenyeres K Studies of the pathogenesis of the high renal clearance of phosphate in hypophosphatemic vitamin D refractory rickets of the simple type A M A J Dis Child 98 586 1959
146 Field M H and Reiss E Vitamin D resistant rickets a disease associated with normal renal transport of phosphate J Clin Invest 38 1004 1959
147 Bessau G and Lohr H Über eine Heilungsmoglichkeit gewisser Vitamin D resistenter Rachitisformen Monatsschr Kinderh 90 1 1942
148 Green W T Discussion of [26] J Bone & Joint Surg 33 A 219 1951
149 Dent C E Harper C M Morgans M E Philpot G R and Trotter W R Insensitivity to vitamin D developing during the treatment of postoperative tetany Its specificity as regards the form of vitamin D taken Lancet 269 687 1955
150 Harrison H C Harrison H E and Park E A Vit D and citrate metabolism Inhibition of Vit D effect by cortisol Proc Soc Exper Biol & Med 96 768 1957
151 Kodicek E Discussion of [135] in *Bone Structure and Metabolism* Ciba Foundation Symposium edited by G E W Wolstenholme and C M O Connor p 201 ff Little Brown Boston 1956
152 Stearns G and Warweg E Studies of phosphorus of blood III The phosphorus partition in whole blood and in serum and the serum calcium and plasma phosphatase during healing of late rickets Am J Dis Child 49 79 1935
153 Guest G M and Rapoport S Role of acid-soluble phosphorus compounds in red blood cells in experimental rickets renal insufficiency pyloric obstruction gastroenteritis ammonium chloride acidosis and diabetic acidosis Am J Dis Child 58 10?? 1939
154 Rapoport S and Guest G M Changes of organic acid soluble phosphorus

diphosphoglycerate adenosinetriphosphate and inorganic pho phorus in the blood cells of rats during the development and healing of rickets J Biol Chem 126 749 1938
155 Guest G M and Rapoport S Organic acid soluble pho phorus of tl e blood Physiol Rev 21 410 1941
156 Rupp W Untersuchungen der Iho pl orsaureester mit Radiophosphor und Papierionophorese Helvet paediat acta 10 11 1955
157 Winters R W McFalls V W and Graham J B A girl with sporadic hypophosphatemia and vitamin D resistant rickets Pediatrics (in press)
158 Shohl A T Fan C H and Farber S Effect of A T 10 (dihydrotachysterol) on various types of experimental rickets in rats Proc Soc Exper Biol & Med 42 529 1939
159 Yendt E R and Howard J E Studies on the mode of action of citrate therapy in rickets Bull Johns Hopkins Hosp 96 101 1955

## Chapter 37

# The Syndrome of Osteomalacia, Renal Glycosuria, Aminoaciduria, and Hyperphosphaturia (The Fanconi Syndrome)

*Alexander Leaf*

The main features of this syndrome upon which there is general agreement are rickets or osteomalacia resistant to vitamin D in usual doses glucosuria generalized aminoaciduria and hyperphosphaturia in spite of normal or reduced plasma concentrations of these constituents and usually chronic acidosis and hypopotassemia Beyond these cardinal features one finds much disagreement and confusion in the literature pertaining to this rare disturbance Recent awareness that multiple etiologies may reproduce these cardinal features and that each feature may occur separately or in various incomplete combinations indicates that the appellation *syndrome* rather than *disease* is appropriate To avoid ambiguity in the present writing eponyms will be avoided and the italicized term *syndrome* will be used to apply to the complete constellation of features as defined above

## HISTORICAL RÉSUMÉ

The history of the recognition of this *syndrome* has been confused by uncertainty as to just which features it encompasses and which eponyms should be applied to it Not all the features recognized today as part of the *syndrome* were appreciated by earlier workers Their case reports therefore seem fragmentary and incomplete making it difficult to trace the historic lineage accurately

In 1903 Abderhalden (*1*) found by chemical assay excessive amounts of cystine in various tissues at post mortem examination of a 21 month old Swiss infant who died of inanition Crystals identified as cystine

were visible macroscopically in the liver and spleen Two siblings had died previously of a similar ailment and the urine of two living siblings contained excessive cystine as did to a lesser extent the urines of the father and paternal grandfather He thus labeled the disorder a familial cystine diathesis

In 1924 Lignac [2 3] a Dutch pediatrician described three similar cases in children 14 months old and 2 and 3 years of age Clinically all three had severe rickets renal disease dwarfism and progressive wasting Pathologically all showed widespread deposits of cystine in the tissues and nephritis, and one child reportedly had cystine stones in the renal pelvis and ureter Lignac also noted the familial incidence of the disease as a sister of one of his patients was afflicted Two further cases were reported from England by Russel and Barrie [4] in 1936 One child had rickets with a low normal serum phosphate concentration but no cystine crystalluria Both he and the second child probably died in uremia since post mortem examination revealed extensive glomerular and tubular damage as well as cystinosis The following year Beumer [5] added albuminuria and glycosuria to the clinical picture and demonstrated in his case that the nonprotein nitrogen level became elevated only terminally Thus cystine storage disease came to be recognized

Meanwhile in 1931 Fanconi [6] briefly described rickets and stunted growth in a child with albuminuria and glycosuria In 1933 deToni [7] reported a dwarfed child with vitamin D-resistant rickets and spontaneous fractures who showed a low serum phosphorus concentration acidosis glycosuria and albuminuria With a similar case reported by Debré [8] in 1934 the one of deToni and two more of his own Fanconi [9 10] in 1936 established a syndrome of nephrotic glycosuric dwarfism with hypophosphatemic rickets Beumer and Wepler [11] in 1937 surmised that cystinosis and the Fanconi syndrome were identical and this seemed proved when at necropsy one of Fanconi s original cases showed cystine deposits in his tissues [12] The extreme form of this view is supported by Bickel and associates [14] who concluded that Lignac s disease (cystine storage disease) and Fanconi s syndrome of childhood (nephrotic glycosuric dwarfism) are one and the same disease and at the present time this disease cannot be identified with similar syndromes in which cystine storage has been excluded Though it is generally accepted today that the two condition are similar this point of view is opposed by deToni [13] who believes that when cystinosis and the syndrome of renal glyco amino-phosphaturia coexist the latter probably represents a complication of the former DeToni would therefore create two separate syndromes the Abderhalden Kaufmann Lignac syndrome to designate cystine storage disease and the deToni Debre-Fanconi syndrome to designate rickets or osteomalacia with renal glyco amino-phosphaturic diabetes The nomenclature is further confused by the use of various

combinations of eponyms for various features of the syndrome Many case reports of this *syndrome* are summarized in the monographs of McCune, Mason, and Clarke [15] Bickel, Smallwood, Smellie, and Hickmans [14], and Eberlein [16]

The *aminoaciduria in the syndrome* was first suspected by Fanconi [10] when he found an increased organic aciduria McCune, Mason and Clarke [15] were the first to demonstrate that the organic aciduria was in fact largely attributable to amino acids

It has become evident that the *syndrome* is not restricted to infancy or childhood but may present initially in adult life with the same cardinal features Cystine deposits in the tissues have not as yet been described in the adult cases although they are commonly an associated finding *in the childhood form* The adult form of the *syndrome* has recently been reviewed by Wallis and Engle [17], who in 1957, collected 18 cases from the literature

## CLINICAL FEATURES

In the infant the disease characteristically begins after a normal post natal period of 4 to 6 months Failure to grow, often bouts of vomiting and unexplained fever, a severe form of rickets resistant to the usual doses of vitamin D chronic acidosis polyuria and dehydration dominate the clinical picture In spite of glucosuria and phosphaturia the blood glucose concentration is not elevated and the serum phosphorus level is usually normal or low, but it may rise terminally with renal glomerular failure In spite of systemic acidosis the urine is relatively alkaline and has a high content of ammonium A generalized aminoaciduria usually involving excessive excretion of 10 or more amino acids, is found [18] *The high cystine excretion is just one facet of the more generalized* aminoaciduria and need not occur even in the presence of large losses of other amino acids This urinary amino acid pattern is therefore quite distinct as are the other clinical features from that of 'cystinuria,' in which excessive urinary losses of only cystine lysine, arginine and ornithine are found (cf Chap 42)

The familial incidence of the condition has been noted and will be examined below The prognosis has generally been poor Few patients *survive more than a few years* but de Toni [19] recently reported ' a case of the syndrome where the patient after having presented grave manifestations in infancy nevertheless reached adult age and may be looked upon as having made a complete recovery *Death is usually due to* progressive renal failure or intercurrent infection Post mortem examination sometimes reveals crystals of cystine extensively deposited throughout the body and cystinosis may be detected during life by *examination* of the conjunctiva and cornea with a slit lamp [20 21] and of the bone

marrow [21 22] As indicated above controversy continues regarding the relationship of cystine storage disease to this *syndrome* Cystine storage definitely does not occur in all instances In 11 recorded autopsies on patients with glycosuria and rickets collected by McCune et al [15] cystine deposits were recognized in 4 and their presence was suspected in a fifth Because cystine crystals may easily be lost in fixation of tissues or may be unrecognized, it is impossible to ascertain the exact incidence of cystine storage at present It seems highly likely that the *syndrome* may occur in the absence of cystinosis in fact, cystinosis has not yet been described in the adult cases

In the adult the *syndrome* has the same features as in the infant, i e, osteomalacia with multiple fractures and pseudofractures hypophosphatemia with hyperphosphaturia usually elevated alkaline phosphatase level generalized aminoaciduria with normal concentrations of amino acids in the serum and renal glucosuria Frequently there are albuminuria alkaline urine and mild or severe systemic acidosis occasionally hypokalemia and hypouricemia are present Crippling bone disease with pain and deformities dominates the clinical picture Glomerular filtration rate may be normal or only moderately reduced but the usual cause of death is glomerular insufficiency with retention of nitrogenous waste products and uremia

## PATHOPHYSIOLOGY

### CALCIUM AND PHOSPHORUS METABOLISM

Rickets or osteomalacia the most prominent clinical feature of the syndrome early focused attention on the derangement of calcium and phosphorus balance

A reduced serum phosphorus concentration has been a common finding although with terminal glomerular failure the level rises to normal or higher The serum calcium levels are usually normal while the alkaline phosphatase concentration is elevated The chemical features of osteomalacia [23] are therefore present in the blood and the bone disease has been repeatedly shown both radiologically and histologically to be rickets in the growing child and osteomalacia in the adult

Hyperphosphaturia has been considered an important feature of this condition [15 24-27] In addition elevated renal clearances of phosphate have been reported [28-30] indicating simply that renal excretion of phosphate remains relatively high despite the low serum phosphate concentration From the available evidence the usual causes of an increased renal clearance of phosphate do not seem to explain the findings in the *syndrome* The usual causes include primary and secondary hyperparathyroidism

Primary hyperparathyroidism seems definitely excluded by the absence

of hypercalcemia [17] and the presence of osteomalacia rather than osteitis fibrosa cystica The reported findings in the parathyroid glands at necropsy are difficult to interpret but tumors have not been described [15], and mention of normal parathyroids has been made [9 10 31] Surgical biopsy specimens of parathyroid glands from two of Hunter s cases were interpreted as normal [32]

Secondary hyperparathyroidism is not so readily excluded, histologically its presence or absence may be very difficult to ascertain By definition this condition refers to a compensatory state requiring persistence of some *physiologic* stimulus to increase parathyroid activity above that normally required to meet the needs of the body Albright and Reifenstein have considered a reduction in the concentration of serum calcium to constitute the effective stimulus [23], resulting in the following sequence of events decreased serum calcium concentration ⟶ increased parathyroid activity ⟶ increased renal clearance of phosphate ⟶ reduced serum phosphate ⟶ return of serum calcium concentration toward normal [1] To establish the presence of secondary hyperparathyroidism it is necessary to demonstrate a cause for a reduced serum calcium level The usual cause is either an increased calcium excretion by the kidneys or a reduced absorption of calcium from the gut However no such impairment in calcium metabolism can be traced in all cases of the *syndrome*

Primary hypercalcinuria has not been noted and values for calcium excretion within a low or normal range are reported [15 28 30] Furthermore the clinical picture bears no resemblance to that of idiopathic hypercalcinuria in which renal stone formation is the cardinal finding [33] Hypercalcinuria is also a feature of renal tubular acidosis [23] and some patients with the *syndrome* have definite hyperchloremic acidosis [15 29] but others have severe osteomalacia with increased phosphate clearance in the complete absence of systemic acidosis [28 31 54] and with normal ability to excrete an acid load [28] Albright and Reifenstein [23] postulated that the increased anion excretion imposed by the aminoaciduria would overtax the hydrogen ion excretory mechanisms (fixed cation-sparing mechanisms) of the kidney so that an increased calcium excretion would result causing demineralization of bone This was an a priori assumption made admittedly without any cases to study This view is disproved by the evidence that the amino acid excretion in the *syndrome* usually contributes only a small additional load of acids to those requiring excretion that severe bone disease may be present when aminoaciduria is a minimal feature [28 34] and that the renal acidifying

[1] The secondary hyperparathyroidism seen in chronic azotemic renal failure need not be considered here since an elevated rather than reduced serum phosphorus level is present The renal clearance of phosphate is decreased not increased and the resulting osteodystrophy is not rickets or osteomalacia [23]

mechanisms of the kidney are not always overtaxed as evidenced by a normal response to further loading with strong acids [28] Thus excessive renal loss of calcium can be excluded as initiating the disturbance in calcium and phosphorus metabolism

An impaired absorption of calcium from the intestines is not a constant finding either A lack of vitamin D in the usual doses is certainly not a factor Treatment of the bone disease with usual or even moderately large doses of vitamin D provides inconspicuous or ephemeral benefit [15] Normal blood levels of vitamin D [35 36] were reported in the case of *Guild Pierce and Lilienthal [24] The normal levels of serum calcium* together with the usually normal or even high levels of urinary calcium excretion [15] would seem to exclude simple failure of intestinal absorption of calcium Not all investigators however agree on the last point Bickel and associates [14] have interpreted their own results and those in the literature as indicating impaired intestinal absorption of calcium as the major cause of the bone disease Recently Davies et al [37] have likewise reported a disturbance in intestinal absorption of calcium and phosphorus together with a very low urinary calcium excretion in a case of the *syndrome*

Kyle and associates [28] have made important observations in an adult with the *syndrome* By means of balance studies they showed (Fig 37 1) the following (1) Without therapy and while on a diet containing 1 250 mg calcium and 1 900 mg phosphorus daily the patient was in negative calcium and phosphorus balance The urinary phosphorus level was high but there were normal amounts of calcium in the urine Serum calcium concentration was normal and serum phosphorus low (2) Alkali therapy with Shohl s solution (sodium citrate—citric acid mixture) had no effect on the calcium or phosphorus balance, a finding consistent with the complete absence of acidosis in their case (3) 100 000 units of vitamin D daily improved calcium balance by decreasing fecal calcium loss but urinary calcium excretion was not reduced and urinary phosphate excretion rose still higher (4) Supplementary intake of neutral phosphate resulted in a positive phosphate balance with increased serum phosphorus concentration although excretion of pho phate in the urine ro e and most significantly there was a *decrease* in urinary calcium excretion In this one case they were therefore able to exclude acidosis vitamin D deficiency and an intestinal absorption defect as the cause of osteomalacia *Suggestive evidence was* further presented that the hyperphosphaturia was not secondary to hyperparathyroidism since the patient responded normally to the intravenous administration of parathyroid hormone with a further large phosphate diuresis This would not be expected had exce ive circulating parathyroid hormone initially been re ponsible for the hyperphosphaturia These metabolic studies thus speak in favor of a primary defect in renal

tubular reabsorption of phosphate, a view suggested repeatedly in the past [10, 16, 27, 31, 35]

The absence of any apparent secondary cause for the increased renal clearance of phosphate had led most workers to postulate a primary defect in the renal tubular conservation of phosphate It should be

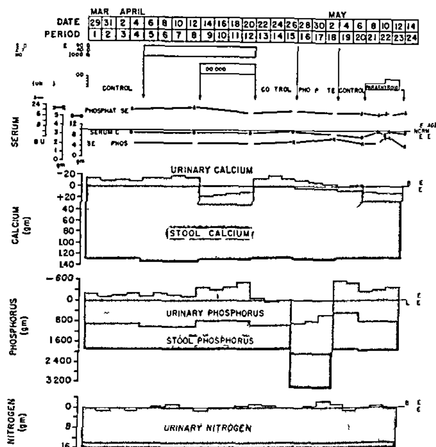



Fig 37 1 Results of the balance study showing calcium phosphorus and nitrogen changes in a 42-year-old male with the *syndrome* (*Data of Kyle Meroney and Freeman [28] reproduced by permission of the Journal of Clinical Endocrinology and Metabolism*)

pointed out however that in the case carefully studied by Milne, Stanbury and Thomson [29] there occurred an apparent decrease in the high renal clearance of phosphate consequent to successful therapy of the bone disease with vitamin D and alkali This finding suggests that the high renal clearance initially present in their case was a secondary rather than a primary defect Correction of acidosis together with improvement of gastrointestinal absorption with vitamin D makes it difficult to evaluate the single clearance they report after therapy since

correction of acidosis alone might have reduced the phosphate clearance The studies presented by Anderson Miller and Kenny [34] also showed a drop in phosphate clearance when therapy with alkali and vitamin D improved calcium and phosphorus balances in their case Thus with an increase in serum phosphorus concentration from about 2 0 to 3 5 mg per 100 ml the urine phosphorus rose only from 1 7 to 1 9 gm per 24 hr Salassa Powers Ulrich and Hayles [39] have confirmed this finding in two patients in whom balance studies were done Whereas alkali administration with correction of acidosis failed to correct the hypophosphatemia or consistently alter the balances of calcium or phosphorus administration of vitamin D in doses of 400 000 units daily for 30 days produced marked improvement in both patients As a result of a marked decrease in fecal phosphorus and only a slight increase in urinary phosphorus during administration of vitamin D the phosphorus balance became positive and the renal clearance of phosphate dropped 37 and 44 per cent, respectively in their two cases

These changes in renal phosphate clearance with repletion of body calcium and phosphorus stores are those classically ascribed to relief of secondary hyperparathyroidism [23] However an initial cause for secondary hyperparathyroidism can not be elicited in all cases of the *syndrome* The alternate postulate that the phosphate loss is due to a primary defect in the renal tubular conservation of phosphate must still be retained This defect perhaps may be specifically corrected by vitamin D in very large doses and indeed large doses of vitamin D may also have a favorable influence on other associated tubular defects (see below) Such a renal tubular defect might be modified in individual cases however by the existence of other factors known to affect calcium and phosphorus metabolism

Davies et al [37] make the interesting suggestion that a disturbance in intestinal as well as in renal tubular reabsorption may exist The high intestinal phosphate would then secondarily interfere with intestinal absorption of calcium to explain the finding of a low urinary calcium excretion in some cases of the *syndrome* Vitamin D according to this view corrects the intestinal as well as the renal defect in phosphate absorption thereby improving the calcium balance together with the phosphorus balance The secondary hyperparathyroidism induced by impaired intestinal absorption of calcium would thus be relieved

## ACIDOSIS AND ASSOCIATED ELECTROLYTE DISTURBANCES

Although not a cardinal feature of the *syndrome* as defined above acidosis is a common finding in both the infantile and adult types In untreated cases the acidosis may be severe The serum $CO_2$ content may fall as low as 9 mEq per liter [14] The disturbances in acid base balance have been carefully studied by Linder, Bull and Grayce [38] in an adolescent

by Milne and associates [29] Kyle and associates [28] and Sirota and Hamerman [30] in adult cases and by Bickel and associates [14] in several cases of the infant and childhood form

The earliest thorough investigation into the acid base metabolism in the *syndrome* was that of Linder Bull, and Grayce [35] Studying a 13-year-old girl they found the following (1) The serum was of normal reaction but with slightly reduced bicarbonate concentration (c 20 mEq per liter) (2) The rate of organic acid excretion was two to three times normal (3) Following administration for 4 days of 2 5 mEq hydrochloric acid per kilogram of body weight per day, chloride excretion rapidly increased but ammonium excretion was distinctly subnormal, and titratable acids did not increase as expected The excretion of the administered chloride was therefore, accompanied by a large loss of fixed cations

*The patient of Milne, when untreated* showed the typical picture of hyperchloremic acidosis The serum bicarbonate was 14 mEq per liter (normal 25 to 30) the serum chloride was 118 mEq per liter (normal, 97 to 103) and the blood pH 7 1 to 7 3 (normal 7 35 to 7 43) The glomerular filtration rate as indicated by inulin clearance was reduced to 31 ml per min, a value approximately one-third to one-quarter the expected normal value The plasma urea concentration was not elevated An abnormal loss of bicarbonate in the urine was demonstrated Even with the serum bicarbonate concentration reduced to 14 mEq per liter the daily excretion of bicarbonate in the urine was 20 mEq Since at this plasma level all bicarbonate filtered is normally reabsorbed [40] the finding constitutes definite evidence for a reduced renal threshold for bicarbonate Such bicarbonate wastage is a known feature of renal tubular acidosis [41] It was not associated with an absolute loss of reabsorptive capacity for bicarbonate reabsorption was found to increase as in the normal person when the serum bicarbonate excretions rose with alkalinizing therapy

The failure of the patient to excrete an acid urine was attributed to the presence of bicarbonate in the urine Only after all the bicarbonate is reabsorbed from the tubular urine does the further secretion of hydrogen ions into the distal tubular urine succeed in making the urine strongly acid When reabsorption of bicarbonate proximally is faulty so that bicarbonate is present in the distal tubular urine hydrogen ion secretion by the distal tubule serves only to convert bicarbonate to carbonic acid As the carbonic acid that is formed undergoes rapid decomposition to volatile carbon dioxide and neutral water the urine is not rendered acid

Although Milne and associates found the rate of ammonium excretion in the urine to be normal or high for the pH of the urine ammonium production was nonetheless totally inadequate in a biologic sense for the degree of systemic acidosis The finding of an ammonium concentra

tion relatively high for the urine pH would at present be ascribed to the concurrent potassium deficiency [47]

The disturbance in acid base regulation noted by Sirota and Hamerman [50] and their studies of this disturbance in their case were very similar to the observations of Milne and associates [29] Their patient also had a hyperchloremic acidosis with urinary wasting of bicarbonate and impaired ammonium production

All 14 of the children with the *syndrome* studied by Bickel and associates [14] were acidotic as indicated by a lowered $CO_2$ combining power of the plasma In each the degree of acidosis varied considerably quite apart from treatment and normal or only slightly lowered $CO_2$ values were found occasionally in almost all Several of their patients were found to excrete large quantities of bicarbonate in the urine despite acidosis with reduced plasma $CO_2$ Defective ammonium formation and titratable acid secretion in some of the children likewise indicated either an inadequate or completely absent response to the administration of acid Organic acid excretion was increased

The findings of the above workers thus indicate a complex disturbance in renal function as the cause of the acidosis with defective tubular reabsorption of bicarbonate playing the major role and defective ammonium production reduced titratable acidity and increased excretion of organic acids all contributing As evidence of normal renal responsiveness to acidosis in this *syndrome* a patient of Fanconi [10] and one of McCune [15] are often cited to show that urine pH may be low while ammonia production and titratable acidity are high In McCune's patient the plasma bicarbonate which was between 10 9 and 18 7 mEq per liter indicated severe acidosis With such reductions of serum bicarbonate one might expect that even with a diminished tubular reabsorptive capacity for bicarbonate all of the decreased amounts of bicarbonate filtered would be reabsorbed thereby permitting production of an acid urine with increased ammonium and titratable acids Thus Bickel et al [14] noted that in one of their patients the bicarbonate loss stopped completely during a period of acid administration at the same time the urine pH dropped from 8 14 to 5 38 while the plasma $CO_2$ fell to the alarmingly low level of 7 3 mEq/l At the present time it may be justifiably concluded that even when the renal hydrogen-excreting mechanisms appear to be functioning normally the response is totally inadequate for the degree of systemic acidosis If recognition is given to the expected absence of bicarbonate in the urine when the patient is severely acidotic it seems that the renal disturbances together with the moderate increase in organic acid excretion mentioned adequately account for the acidosis

Ketonuria has been a common finding in this *syndrome* It is not surprising in view of the renal glucosuria and inanition but has not been

proved in any instance to be of quantitative importance in producing acidosis

The impression should not be left that acidosis or impaired renal mechanisms for hydrogen ion secretion are essential parts of the disease process In the well-documented case of Kyle et al [28] the patient had a normal plasma bicarbonate and in response to an acid load of 130 mEq ammonium chloride daily for 5 days produced as acid a urine with as high a titratable acidity as did a normal control similarly treated and an even higher ammonium content than the control The bone disease and other features of the *syndrome* were nevertheless all present The variability in the number and severity of the features apparent in any given case will be considered further below

A deficiency of potassium which is causally related to the defect in hydrogen ion excretion has been generally appreciated since the study of Milne et al [29] although Linder and associates previously [38] commented on the loss of sodium and potassium in association with the production of dehydration and symptoms of potassium deficiency Case histories of this *syndrome* refer frequently to unexplained bouts of weakness, paralysis and collapse An interesting occurrence termed "dextrose shock" has been noticed This consists of severe and sudden peripheral collapse during sugar tolerance tests Both Debre [8] and Fanconi [10] witnessed such episodes in conjunction with the oral administration of dextrose for glucose tolerance tests Debré s patient became restless and pallid and then lapsed into fatal coma In neither of the two patients of Fanconi did the episode prove fatal These episodes are most probably the result of hypokalemia a finding repeatedly noted in this *syndrome* [14 17, 29] In addition to the weakness paralysis and collapse potassium depletion is undoubtedly responsible for at least two other common findings polyuria and at post mortem, vacuolar changes affecting the renal tubular epithelium Polyuria with isosthenuria had been frequently noted and in some instances shown to be resistant to antidiuretic hormone [38] This is characteristic of the nephropathy of potassium deficiency [42] Recently Davies et al [37] have demonstrated improvement in urinary acidification following correction of potassium deficiency in their patient They considered the potassium depletion to be primary rather than secondary to the disturbance in acid base regulation and hence to represent a separate tubular reabsorptive defect

## GLUCOSURIA

Glycosuria was recognized early in the delineation of this *syndrome* and has remained one of its most definite and least controversial features The reducing substance in the urine was found to be glucose by Fanconi [9 10] and subsequently by others It is of renal origin i e the result of a lowered renal threshold for glucose In the few instances in which diabetic

like glucose tolerance test results have been observed preceding carbohydrate depletion was probably responsible In many instances cases have been incorrectly diagnosed and treated with insulin The glucosuria is variable, ranging from mere traces to 4 to 5 gm per 100 ml [14 15]. The elimination of sugar is characteristically continuous at least in the more severe forms of the *syndrome* but it may be intermittent and not infrequently will disappear terminally

The presence of glucosuria has had a special significance in this *syndrome* in helping to localize the site in the nephron where the functional impairment resides The findings of Richards [43] are generally accepted as demonstrating that glucose reabsorption occurs in the proximal tubule Hence the presence of renal glucosuria has constituted a strong argument in favor of placing the disturbance in the proximal tubule The location of the disturbance in the kidney will be considered in greater detail below

## AMINOACIDURIA

Aminoaciduria is the most recently recognized feature of the *syndrome* *Although suspected by Fanconi [10] it was not proved until the work of* McCune and associates [15] It has been investigated in detail only since the techniques of chromatography were applied to the problem notably by Dent [18] who since 1947 in a series of studies [18 44–46] has characterized the main features of the aminoaciduria

The normal individual excretes some 200 to 400 mg amino acids daily [47] Amino nitrogen constitutes less than 3 per cent of the total urinary nitrogen [18] Figure 37 2A and B show the urinary pattern of amino acids from a normal infant and adult respectively The trace amounts *of amino acids in normal subjects are to be contrasted with the high* excretion rates shown by a child and adult with the *syndrome* (Fig 37 3A and B respectively) These figures illustrate the main features of the aminoaciduria (1) It is a generalized aminoaciduria involving usually 10 or more amino acids (2) The pattern of amino acid excretion is variable from case to case but seems to remain quite constant with time in any individual case [45] (3) Cystine excretion may be increased or normal even in the presence of cystinosis Renal calculi of cystine have so far been reported in only one case [48] These features distinguish the *aminoaciduria* sharply from that seen in cystinuria in which the excretion of only four amino acids is increased and these are always the same (cystine lysine arginine and ornithine) [49–51] as shown in Fig 37-4

The generalized aminoaciduria is reminiscent of the overflow type seen in severe liver disease [52 53] However Dent has demonstrated that the plasma amino acid concentrations are normal or low [18] rather than elevated as in liver disease Harper and associates [54] have also reported essentially normal plasma levels for several amino acids that were present

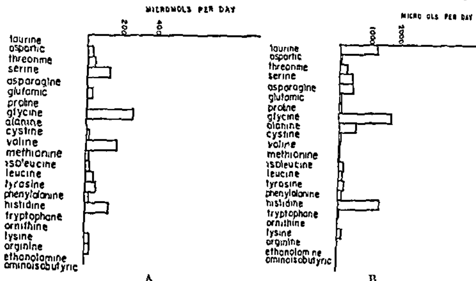



Fig 37 2 A The daily amino acid excretion in the urine of a normal infant (*According to Jonxis* [73]) B The daily amino acid excretion in the urine of a normal adult (*Data of Harrison and Harrison* [83] *reproduced by permission of the Journal of the American Medical Association*)

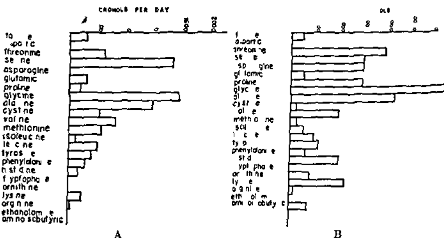



Fig 37 3 A The daily amino acid excretion in the urine of a child with cystine storage disease and the *syndrome* (*From Harrison and Harrison* [83]) B The daily amino acid excretion in the urine of an adult with the *syndrome* (*Data of Evered* [47] *recalculated by Harrison and Harrison* [83] *and reproduced by permission of the Journal of the American Medical Association*)

in excess in the urine Although Dent s findings were contested by Bickel and Smellie [21] who reported levels 50 to 100 per cent above the normal range Evered has recently [47] reexamined the problem with the more precise method of quantitative ion-exchange column chromatography and has convincingly supported Dent s results Thus the renal clearance

of amino acids i high in the *syndrome* and a defect in renal tubular reabsorption would seem to be responsible for this feature

Because knowledge regarding the normal tubular mechanisms for amino acid transport is only fragmentary the defect responsible for the aminoaciduria in the *syndrome* is not understood Lathem Baker and Bradley [55] found a gross aminoaciduria during fastings in their patient with the *syndrome* However during an intravenous infusion of amino acids (hydrolyzate of fibrin) the increase in amino acids in the urine was not in excess of that occurring normally during a similar infusion They suggested that the defect in tubular reabsorption of amino acids may involve only a small percentage of the nephron population This conclusion is at variance with both the reported widespread distribution of the morphologic changes in the kidney (see below) and the results of Robson and Rose [56] who found a high initial clearance of cystine in their patient which rose only slightly following infusion of lysine to equal the clearance of inulin Beyer and associates [57] have studied competition for reabsorption of various amino acids by the renal tubules and indicated that at least three separate transport systems are present in the dog for tubular reabsorption of different amino acids If these studies in the dog are applicable to the human kidney patients with the *syndrome* lose amino acids from all three groups Pitts [58] in studies also with the dog demonstrated that the reabsorptive processes of the tubules of amino acids are distinct from those involved in glucose absorption

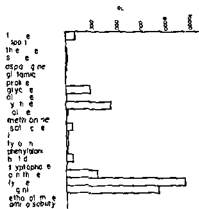


Fig 37-4 Urinary excretion of amino acids by a patient with cystinuria (*Data of Ever d [4 ] recalculated by Harrison and Harrison [80] and reproduced by permission of the Journal of the American Medical Association*)

## MORPHOLOGIC CHANGES

Only recently has any lesion been described at necropsy which appears to have some specificity to this disorder There are reasons to believe that a lesion in the proximal renal tubule could most easily account for the renal disturbances of the *syndrome* Thus there has been great interest in the report in 1953 of Clay Darmady and Hawkins [59] of a characteristic lesion affecting this portion of the nephron By using a micro dissection technique to isolate individual nephrons these workers ob

served two apparently characteristic types of lesions present in the kidneys of two patients with the *syndrome* (1) The swan neck deformity illustrated in Fig 37 5B, was present 100 times out of 101 consecutive nephrons examined at random In this lesion normal glomeruli are attached to a narrow neck in which the epithelium is of a low cuboidal form for a distance of 0 3 to 1 0 mm Further down the epithelium suddenly expands into the height and shape of the normal proximal tubule (Fig 37 5A) but differs from it in possessing a greater translucency (2) The second change is a shortening of the whole proximal convoluted




Fig 37-5 A Normal human glomerulus and proximal tubule as seen following microdissection B Glomerulus and nephron from a patient with the *syndrome* showing the swan neck lesion affecting the proximal tubule C Glomerulus and nephron from a patient with the *syndrome* showing the atrophy and shortening of the proximal tubule (*From Darmady and Stranack [60] reproduced by permission of the British Medical Bulletin* )

tubule including its narrow portion The authors considered that the proximal tubules were atrophic and about one-fourth to one half the normal length This lesion is also shown in Fig 37 5C in which a normal nephron has been included for comparison

More recently Darmady and Stranack [60] have reported their further experience with the microdissection technique in 11 cases " where the clinical history conformed strictly to that laid down for Lignac Fanconi disease (cystinosis) In 8 the proximal tubule was shorter than normal and its first portion was replaced by a thin and narrow neck for about one third of its length i e the swan neck defect The remaining 3 cases showed a hypotrophic lesion throughout the length of the proximal tubule In the recent report two additional patients are mentioned who in life had glycosuria phosphaturia hypopotassemia abnormal amino aciduria and dwarfism but who lacked cystinosis Both these patients showed the typical swan neck lesion It is of special interest that one of these two patients aged 46 had the adult form of the *syndrome* while

the remaining 12 were all of the infantile or childhood form, the oldest being 9 years of age

The absence of any associated regenerative changes in the kidneys together with the regularity and frequency of the proximal tubular lesions led the authors to the conclusion that these represented con genital defects even though the clinical syndrome often did not become manifest for some time after birth The lesions seemed specific They were not found in tissue obtained from a case of renal amyloidosis and were distinguished from a somewhat similar lesion described by Oliver [61] in chronic nephritis in which only a small fraction of the nephrons are affected

Other renal lesions have been described in this *syndrome* which do not seem to be specific Vacuolation of the tubular epithelium has been noted [15] but this is attributable to the associated potassium depletion which is often encountered in these patients and which is known to produce similar vacuolar changes [41] Atrophic changes in the distal tubular epithelium similar to those seen in acute tubular necrosis have also been reported [59] as have varying degrees of renal fibrosis Since progressive renal impairment with uremia is a common outcome of this *syndrome* fibrosis and loss of nephrons are perhaps to be expected at necropsy but their relationship to the structural basis of the observed renal dysfunction is difficult to ascertain Pyelonephritis may be responsible for the progressive renal failure Potassium depletion may in turn predi pose to pyelonephritis [62] although this suggestion requires further sup port [42]

In addition to renal pathologic changes changes in other tissues have also been noted Skeletal changes consistent with rickets or osteomalacia occur as expected [6] Fatty liver and focal necrosis of the liver or cir rhosis have been noted repeatedly though not invariably Their relation ship to the remainder of the *syndrome* remains obscure [15 27] It has been suggested that the urinary amino acid loss might be the cause of the cirrhosis [27] but evidence is lacking that such loss is of sufficient mag nitude to be considered seriously as a cause of protein depletion Cystine crystals have been reported in the tissues of many but certainly not in all the autopsy reports The fact that Bickel and Smellie [21] were able to demonstrate cystine crystals ante mortem in bone marrow biopsies from all 17 of their cases suggests that a more careful search will reveal crystals in a larger fraction of cases

## ETIOLOGY AND GENETICS

Fanconi is generally credited with the view that impairment of the reabsorptive capacity of the renal tubules without a corresponding reduc tion in glomerular filtration volume could account for the major features

of the *syndrome* A tubular reabsorptive defect seems to be directly responsible for the aminoaciduria glucosuria phosphaturia and the tendency to excrete an alkaline urine, and secondarily responsible for the systemic acidosis and skeletal changes The anatomic changes found by Darmady and associates [59 60] appear to provide a morphologic basis in the proximal tubule for such a defect The proximal tubule is thought to be the site of reabsorption of glucose [43] and Fanconi [63] considers the proximal tubule to be the site of reabsorption of amino acids and phosphate as well Hence the morphologic changes are to this extent consistent with the functional derangements

When one asks the important next question namely what is the cause of the renal tubular defect a unitarian approach to the *syndrome* is no longer tenable The familial occurrence of this rare *syndrome* early suggested an hereditary basis for the disease Recently however, the renal defects associated with the *syndrome* have been recognized repeatedly under circumstances in which renal damage was clearly acquired Harrison and Harrison [64] reported that injections of maleic acid into rats may produce renal glucosuria phosphaturia and a generalized aminoaciduria It had previously been demonstrated that this compound interfered with the renal excretion of acid by acidotic dogs [65] These renal disturbances proved reversible Spencer and Franglen [66] have reported gross glucosuria and aminoaciduria without elevated blood glucose or amino acid concentrations occurring transiently during the recovery phase of acute renal failure in a patient who suffered an extensive Lysol burn Experimental uranium poisoning may produce glucosuria and aminoaciduria [67 68] Gross renal aminoaciduria has been observed in lead poisoning [69–71] and in cadmium mercury and uranium poisoning [72] In at least one instance of lead poisoning in a child [70] the aminoaciduria was associated with glycosuria persistent hypophosphatemia and rickets The acquired *syndrome* in this child resolved after treatment of the lead poisoning Vitamin D deficiency has been associated with renal aminoaciduria [73] Of considerable interest is the association of multiple myeloma and the adult form of the *syndrome* reported recently by Engle and Wallis [74] and noted earlier by Sirota and Hamerman [30] These clinical and experimental observations indicate that the *syndrome* may have multiple etiologies in which the common denominator is renal tubular injury with impairment especially of the systems that are important in the renal tubular handling of amino acids glucose and phosphate

The hereditary nature of this *syndrome* was first noted by Abderhalden [1] and has been referred to repeatedly since Duplication of the *syndrome* in siblings and blood relationship between parents have been noted [15] Dent and Harris [75] concluded from their study of the genetics of this *syndrome* that ' the simplest view is that we are concerned with a simple

recessive character The difficulty of characterizing genetically transmitted traits in human disease as either simply dominant or recessive has been recently emphasized [76] What is definite however is that this rare *syndrome may affect more than one sibling and that there is a high* instance of parental consanguinity [15 75] Classification of the genetic mode of transmission is made difficult because acquired renal tubular damage can mimic this *syndrome* There also remains uncertainty as to whether individuals who develop the adult form of the *syndrome* have had a renal tubular defect since birth or whether the lesion itself developed only in adult life and accordingly whether the infantile and adult forms are the same disease Dent and Harris [75] described an interesting family in which three adult siblings had biochemical abnormalities closely similar to those found in the propositus with the adult form of the *syndrome* Each sibling resembled the patient in having generalized aminoaciduria mild acidosis and hypophosphatemia but unlike their afflicted sister they were clinically well with no evidence of bone disease How long these siblings had had these biochemical disturbances and whether they might have developed clinically apparent disease with time remained unanswerable The biochemical defects could conceivably be present from birth even in the adult cases but not clinically manifest unless the patient was confronted by environmental factors e g pregnancy and lactation which would place a strain on the impaired mechanism for calcium and phosphorus conservation On the other hand adult cases have been reported in which a retrospective search for glycosuria on preceding urinalyses have yielded negative results [75] Even in the infantile form Bickel has reported well-documented cases indicating that the abnormality in renal function was not manifest during the first few months after birth [77]

Dent has pointed out [45] that in the hereditary diseases generally which include some forms of aminoaciduria all the cells of the body carry the defective gene while only in the kidney in the *syndrome* does the abnormality resulting from the defective gene give rise to clinically recognizable disturbances In this regard the association of cystine storage with the *syndrome* in some instances remains a confusing feature Most workers accept the results of Dent [18] and of Evered [47] referred to above which indicate no elevation of plasma amino acid levels in the *syndrome* although Bickel claims that elevated plasma levels may be present [14 77] If elevated plasma amino acid levels are not present some local factor which is not essential to the development of the *syndrome* must be responsible for the widespread deposition of cystine crystals seen in patients with cystinosis DeToni's view [13] that cystinosis and the *syndrome* are different entities whose coexistence is the result of an acquired renal tubular lesion producing the *syndrome* as a complication of cystine storage may have merit This view has also been stated by

of the *syndrome* A tubular reabsorptive defect seems to be directly responsible for the aminoaciduria glucosuria phosphaturia and the tendency to excrete an alkaline urine and secondarily responsible for the systemic acidosis and skeletal changes The anatomic changes found by Darmady and associates [59, 60] appear to provide a morphologic basis in the proximal tubule for such a defect The proximal tubule is thought to be the site of reabsorption of glucose [43], and Fanconi [63] considers the proximal tubule to be the site of reabsorption of amino acids and phosphate as well Hence the morphologic changes are to this extent consistent with the functional derangements

When one asks the important next question namely what is the cause of the renal tubular defect a unitarian approach to the *syndrome* is no longer tenable The familial occurrence of this rare *syndrome* early suggested an hereditary basis for the disease Recently, however the renal defects associated with the *syndrome* have been recognized repeatedly under circumstances in which renal damage was clearly acquired Harrison and Harrison [64] reported that injections of maleic acid into rats may produce renal glucosuria phosphaturia, and a generalized amino aciduria It had previously been demonstrated that this compound interfered with the renal excretion of acid by acidotic dogs [65] These renal disturbances proved reversible Spencer and Franglen [66] have reported gross glucosuria and aminoaciduria without elevated blood glucose or amino acid concentrations occurring transiently during the recovery phase of acute renal failure in a patient who suffered an extensive Lysol burn Experimental uranium poisoning may produce glucosuria and aminoaciduria [67 68] Gross renal aminoaciduria has been observed in lead poisoning [69–71] and in cadmium mercury and uranium poisoning [72] In at least one instance of lead poisoning in a child [70] the aminoaciduria was associated with glycosuria persistent hypophosphatemia and rickets The acquired *syndrome* in this child resolved after treatment of the lead poisoning Vitamin D deficiency has been associated with renal aminoaciduria [73] Of considerable interest is the association of multiple myeloma and the adult form of the *syndrome* reported recently by Engle and Wallis [74] and noted earlier by Sirota and Hamerman [30] These clinical and experimental observations indicate that the *syndrome* may have multiple etiologies in which the common denominator is renal tubular injury with impairment especially of the systems that are important in the renal tubular handling of amino acids, glucose and phosphate

The hereditary nature of this *syndrome* was first noted by Abderhalden [1] and has been referred to repeatedly since Duplication of the *syndrome* in siblings and blood relationship between parents have been noted [15] Dent and Harris [75] concluded from their study of the genetics of this *syndrome* that the simplest view is that we are concerned with a simple

*drome*) To this list should probably be added impaired tubular reabsorption of bicarbonate as seen in renal tubular acidosis [*41*] which frequently occurs in the *syndrome* as discussed above Dent [*44*] had earlier proposed a somewhat similar subdivision of possible conditions arising from combinations of separate tubular reabsorptive defects Thus it is clear that the *syndrome* constitutes no clear-cut entity with distinct clinical functional or etiologic boundaries but merges into the spectrum of disturbances arising from all possible renal tubular defects

## TREATMENT

The problem of management of a condition whose clinical features are the result of renal losses of certain body constituents would seem to be one primarily of replacement therapy The renal losses of glucose or of amino acids in this condition have not constituted a problem because they are quantitatively not sufficient to produce nutritional disturbances Acidosis and phosphate loss require treatment in order to heal the skeletal lesions The rickets and osteomalacia have repeatedly been found to be resistant to usual amounts of vitamin D but improvement in skeletal and biochemical disturbances occurs with large doses of vitamin D (50 000 to 400 000 I U daily) [*21 38 39 44 82*] It is of special interest that the aminoaciduria may be diminished although not necessarily restored to normal values by large doses of vitamin D [*83*]

In view of the excessive renal clearance of phosphate it would seem rational to supplement the dietary intake of phosphate and to replace the excessive loss Until the skeleton has remineralized supplementary calcium may also be needed but a high calcium intake together with large doses of vitamin D considerably increase the risk of hypercalcemia and should be undertaken only if frequent and careful follow up determinations of serum and urine calcium levels are possible

The acidosis has been treated successfully with the solution of Shohl [*30 34*] which contains 140 gm citric acid and 98 gm sodium citrate per liter Since potassium deficiency is commonly present potassium bicarbonate or citrate may be necessary However as chronic acidosis is presumably the cause of the potassium depletion once acidosis has been relieved no further excessive potassium losses should be incurred and supplementary potassium should not be continuously required

Combinations of the above replacement therapy have increased considerably the optimism with which immediate prognosis in this *syndrome* may be regarded Since therapy merely replaces losses rather than cures the tubular defect it is evident that therapy must be continued indefinitely Replacement therapy does not seem to prevent the slow progression of renal impairment with ultimate death from glomerular failure and uremia [*84*] Since terminal renal failure may perhaps occur more

Harrison [78] No one has yet produced a satisfactory explanation of how a renal tubular defect resulting in loss of amino acids can simultaneously result in excessive deposition of cystine in the tissues Clearly some factor in addition to the renal lesion must be present in these cases

The findings of Salassa, Powers, Ulrich and Hayles [39], who carefully studied the effects of large doses of vitamin D (400 000 units daily) for a period of 30 days in two patients deserve special mention Not only did the balances of calcium and phosphorus improve markedly with a reduction in renal clearance of phosphate, but all other parameters that were studied likewise improved Thus the aminoaciduria decreased glucosuria diminished, serum bicarbonate concentrations rose to normal, and serum chloride levels fell to normal The decrease in aminoaciduria occurred in the absence of a detectable change in the plasma concentration of amino acids Curiously, the acidosis corrected itself in spite of a considerable decrease in urinary ammonium excretion Presumably a more acid urine with a high titratable acid excretion must have resulted from the vitamin D but in the one case where titratable acid excretion rates were reported, no increase was found These interesting results require confirmation in a larger number of cases The fact that vitamin D deficiency is associated with an aminoaciduria and that the aminoaciduria is corrected by administration of vitamin D [73] supports at least some of the findings of Salassa and associates Perhaps some processes in the renal tubule which normally are dependent upon vitamin D are defective but in some cases at least are partially correctable by huge doses of vitamin D Much more work will be required before knowledge of the normal tubular reabsorptive mechanisms and their relationship to the action of vitamin D will permit more than conjectures on the etiology of this condition

The arbitrary status of this *syndrome* has been emphasized by recent reports in which all possible combinations of the glucosuria phosphaturia and aminoaciduria have been observed Thus Luder and Sheldon [79] reported A Familial Tubular Absorption Defect of Glucose and Amino Acids in which only glucose and amino acid reabsorption were impaired This defect appeared to be the result of a dominant genetic characteristic They considered this defect to be one of seven possible combinations of defects deriving from abnormalities of tubular reabsorption of phosphate glucose and amino acids (1) glucose alone (as in renal glucosuria) (2) phosphate alone (possibly vitamin D-resistant hypophosphatemic rickets [44]) (3) amino acids alone (cystinuria or hepatolenticular degeneration [80 81]) (4) glucose with phosphate (combination of renal glucosuria and hypophosphatemic rickets [44]) (5) phosphate and amino acids (aminoaciduria in vitamin D deficiency [73]) (6) glucose and amino acids [79] (7) phosphate glucose and amino acids (the syn

7 DeToni G Remarks on the relations between renal rickets (renal dwarfism) and renal diabetes Acta paediat 16 479 1933
8. Debre R Marie J Cléret F and Messimy R Rachitisme tardif coexistent avec une néphrite chronique et une glycosurie Arch de méd d enf 37 597 1934
9 Fanconi G Der nephrotisch glykosurische Zwergwuchs mit hypophosphatamischer Rachitis Deutsche med Wchnschr 62 1169 1936
10 Fanconi G Der fruhinfantile nephrotisch glykosurische Zwergwuchs mit hypophosphatamischer Rachitis Jahrb Kinderh 147 299 1936
11 Beumer H and Wepler W Über die Cystinkrankheit der ersten Lebenszeit Klin Wchnschr 16 8 1937
12 Looser R Ein Fall von Cystinspeicherung mit renalem Zwergwuchs und Rachitis Ann paediat 163 251 1944
13 DeToni G Renal rickets with phospho gluco amino renal diabetes Ann paediat 187 42 1956
14 Bickel H Smallwood W C Smellie J M and Hickmans E M Cystine storage disease with aminoaciduria and dwarfism (Lignac Fanconi disease) clinical description factual analysis and treatment of Lignac Fanconi disease Acta paediat (Suppl 90) 42 27 1952
15 McCune D J Mason H H and Clarke H T Intractable hypophosphatemic rickets with renal glycosuria and acidosis (the Fanconi Syndrome) Am J Dis Child 65 81 1943
16 Eberlein W R Aminoaciduria in childhood cystinuria and cystinosis Am J M Sc 225 677 1953
17 Wallis L A and Engle R. L Jr The adult Fanconi syndrome Am J Med 22 13 1957
18 Dent C E The amino aciduria in Fanconi syndrome A study making extensive use of techniques based on paper partition chromatography Biochem J 41 240 1947
19 DeToni G and Durand P Guarigione clinica del diabete renale e del rachitismo renal in un grave case di sindrome di deToni Debré-Fanconi giunto all età adulta con persistenza del nanismo e della iperaminoaciduria Minerva pediat 7 1053 1955
20 Burki E Ueber Augenveranderungen bei der Cystinkrankheit Ann paediat 156 321 1941
21 Bickel H and Smellie J M Cystine storage disease with aminoaciduria Lancet 1 1093 195
22 Esser M Zur Diagnose der Cystinkrankheit Ann paediat 156 344 1941
23 Albright F and Reifenstein E C Jr *The Para thyroid Glands and Metabolic Bone Disease* Williams & Wilkins Baltimore 1948
24 Guild H G Pierce J A and Lilienthal J L An unfamiliar rachitic syndrome Am J Dis Child 54 1186 1937
25 Goldman F and Eckstein G Renal dwarfism due to tubular dysfunction clinical and metabolic studies of a case J Pediat 15 53 1939
26 Gittleman I F and Pincus J B Rickets associated with dwarfism glycosuria ketonuria and albuminuria Am J Dis Child 60 1351 1940
27 Stowers J M and Dent C E Studies on the mechanism of the Fanconi syndrome Quart J Med 16 275 1947
28 Kyle L H Meroney W H and Freeman M E Study of the mechanism of bone disease in hypophosphatemic glycosuric osteomalacia J Clin Endocrinol 14 365 1954
29 Milne M D Stanbury S W and Thomson A E Observations on the Fanconi syndrome and renal hyperchloremic acidosis in the adult Quart J Med 21 21 1952
30 Sirota J H and Hamerman D Renal function studies in an adult subject with Fanconi syndrome Am J Med 16 138 1954
31 Cooke W T Barclay J A Govan A D T and Nagley L Osteoporosis associated with low serum phosphorus and renal glycosuria Arch Int Med 80 147 1947

readily in the potassium-deficient subject [62], any evidence of urinary tract infection should be carefully investigated and specifically treated

In contrast to 'cystinuria,' cystine excretion in the urine may be either normal or only moderately increased Nephrolithiasis due to cystinuria in this *syndrome* has been reported so far on only one occasion [48] and no specific measures are ordinarily required for its prevention

Those who claim cures for the *syndrome* must take cognizance of the acquired forms secondary to reversible renal tubular damage from a wide variety of agents

## SUMMARY


1 A syndrome characterized by rickets or osteomalacia with renal glucosuria aminoaciduria phosphaturia and frequently acidosis has been reviewed This syndrome may appear early in life (infantile form) or manifest itself first during later life (adult form)

2 The associated physiologic disturbances are best explained as consequences of a defective renal tubular reabsorption of glucose, amino acids phosphate and bicarbonate

3 A characteristic morphologic change has been described in the proximal tubule of these subjects which provides a plausible anatomic basis for the functional disturbances

4 The clinical and experimental observations which have been reviewed indicate that the *syndrome* may have multiple etiologies in which the common denominator is injury to the proximal renal tubule with impairment of its normal reabsorptive functions Thus acquired forms of the *syndrome* are now recognizable in addition to the genetically determined forms which were initially described and in which the hereditary factor acts like a simple recessive character

5 Rational therapy aimed at replacing abnormal renal losses is outlined Reversal of the morphologic changes in the kidney once they have developed seems unlikely and no way of halting the gradual development of glomerular failure and uremia is known at present


## BIBLIOGRAPHY


1 Abderhalden F Familiare Cystindiathese Ztschr physiol Chem 38 557 1903
2 Lignac G O E Ueber Storung des Cystinstoffwechsel bei Kindern Deutsches Arch klin Med 145 139 1924
3 Lignac G O E Derangement of cystine metabolism in children Nederl tijd chr geneesk 68 2987 1924
4 Russel D S and Barrie H J Storage of cystine in the reticuloendothelial system and its association with chronic nephritis and renal rickets Lancet 2 899 1936
5 Beumer H Über die Cystinkrankheit Monatsschr Kinderh 68 251 1937
6 Fanconi G Die nicht diabetischen Glykosurien und Hyperglykamien des alteren Kindes Jahrb Kinderh 133 257 1931

59 Clay R D Darmady E M and Hawkins M The nature of the renal lesion in the Fanconi syndrome J Path Bact 65 551 1953
60 Darmady E M and Stranack F Microdissection of the nephron in disease Brit M Bull 13 21 1957
61 Oliver J *Architecture of the Kidney in Chronic Bright s Disease* Hoeber New York 1939
6 Milne M D Muehrcke R C and Heard B E Potassium deficiency and the kidney Brit M Bull 13 15 1957
63 Fanconi G Beitrage zur Nierenpathologie Acta paediat 40 403 1951
64 Harrison H E and Harrison H E Experimental production of renal glycosuria phosphaturia and aminoaciduria by injection of maleic acid Science 120 606 1954
65 Berliner R W Kennedy T J Jr and Hilton J G Effect of maleic acid on renal function Proc Soc Exper Biol & Med 75 791 1950
66 Spencer A G and Franglen G T Gross aminoaciduria following a lysol burn Lancet 1 190 1952
67 Rothstein A and Berke H Aminoaciduria in uranium poisoning The use of the aminoacid nitrogen to creatinine ratio in spot samples of urine J Pharmacol 96 1 9 1949
68 Voegtlin C and Hodge H C *Pharmacology and Toxicology of Uranium Compounds* McGraw Hill New York 1949
69 Wilson V K Thomson M L and Dent C E Aminoaciduria in lead poisoning Lancet 2 66 1953
0 *Chisolm J M Jr Harrison H C Eberlein W R and Harrison H E* Aminoaciduria hypophosphatemia and rickets in lead poisoning A M A J Dis Child 89 159 1955
71 Marsden H B and Wilson V K Lead poisoning in children Brit M J 1 324 1955
Clarkson T W and Kench J E Urinary excretion of amino acids by men absorbing heavy metals Biochem J 62 361 1956
73 Jonxis J H P Aminoaciduria and rickets Helvet pa diat acta 10 24 1955
74 Engle R L Jr and Wallis L A Multiple myeloma and the adult Fanconi syndrome Am J Med 22 5 1957
75 Dent C E and Harris H The genetics of cystinuria Ann Eugenics 16 60 1951
6 Allison A C and Blumberg B S Dominance and recessivity in medical genetics Am J Med 25 933 1958
77 Bickel H Die Entwicklung der biochemischen Lasion bei der Lignac Fanconis chen Krankheit Helvet paediat acta 10 259 1955
8 Harrison H E The Fanconi syndrome J Chron Dis 7 346 1958
9 Luder J and Sheldon W A familial tubular absorption defect of glucose and amino-acids Arch Dis Childhood 30 160 1955
80 Cooper A M Eckhardt R D Faloon W W and Davidson C S Investigation of the aminoaciduria in Wilson s disease (hepatolenticular degeneration) demonstration of defect in renal function J Clin Invest 29 65 19 0
81 Uzman L L and Hood B Familial nature of aminoaciduria in Wilson s disease (hepatolenticular degeneration) Am J M Sc 223 39 19 2
82 Dragstedt I J and Hjorth N Fanconi syndrome (osteomalacia due to decreased renal resorption of phosphate with other tubular functional defects) Acta med Scand 146 317 1953
83 Harrison H E and Harrison H C Aminoaciduria in relation to deficiency diseases and kidney function J A M A 164 1571 1957
84 Case Records of the Massachusetts General Hospital Case 44341 New England J M d 259 39 1958

32 Hunter D Studies in calcium and phosphorus metabolism in generalized diseases of bone Proc Roy Soc Med 28 1613 1935

33 Henneman I H Benedict P H Forbes A I and Dudley H R Idiopathic hypercalciuria New England J Med 259 802 1958

34 Anderson I A Miller A and Kenny A Osteomalacia and renal glycosuria in adults Quart J Med 21 33 1952

35 Warkany J Estimation of vitamin D in blood serum Am J Dis Child 52 831 1936

36 Warkany J and Mahon H E Estimation of vitamin D in blood serum II Level of vitamin D in human blood serums Am J Dis Child 60 606 1940

37 Davies H E F Evans B Rees H M N and Fourman P The defective *absorption of phosphorus and calcium and the effect of vitamin D in the Fanconi* syndrome Guy s Hosp Rep 107 486 1958

38 Linder G C Bull G M and Grayce I Hypophosphatemic glycosuric rickets (Fanconi syndrome) Clin Proc 8 1 1949

39 Salassa R M Powers M H Ulrich J A and Hayles A B Observations on the metabolic effects of vitamin D in Fanconi s syndrome Proc Staff Meet *Mayo Clin 29 214 1954*

40 Pitts R F Ayer J L and Schiess W A The renal regulation of acid base balance in man III The reabsorption and excretion of bicarbonate J Clin Invest 28 35 1949

41 Latner A L and Burnard E D Idiopathic hyperchloraemic renal acidosis of infants Quart J Med 19 285 1950

42 *Relman A S and Schwartz W B The kidney in potassium depletion* Am J Med 24 764 1958

43 Richards A N The Croonian Lecture Processes of urine formation Proc Roy Soc London s B 126 398 1938

44 Dent C E Rickets and osteomalacia from renal tubule defects J Bone & Joint Surg 34B 266 1952

45 Dent C E *Chromatography in the Study of Amino Acid Metabolism Lectures on the Scientific Basis of Medicine* vol 2 The Athlone Press London 1954

46 Dent C E and Harris H Hereditary forms of rickets and o teomalacia J Bone & Joint Surg 38B 204 1956

47 Evered D F The excretion of amino acids by the human A quantitative study with ion-exchange chromatography Biochem J 62 416 1956

48 Lignac G O E *Cystinbefunde bei einer bestimmten* Kinderkrankheit Verhandl deutsch Gesellsch Path 21 303 1926

49 Yeh H L Frankl W Dunn M S Parker P Hughes B and Gyorgy P The urinary excretion of amino acids by a cystinuric subject Am J M Sc 214 507 1947

50 Stein W H Excretion of amino acids in cystinuria Proc Soc Exper Biol & Med 78 705 1951

51 Dent C E and Harris H The genetics of cystinuria Ann Eugenics 16 60 1951

52 Dent C E Mechanisms of aminoaciduria Fed Proc 6 390 1947

53 Walshe J M Disturbances of amino acid metabolism following liver injury study by means of paper chromatography Quart J Med 22 483 1953

54 Harper H A Grossman M Henderson P and Steinbach H Renal amino aciduria A M A J Dis Child 84 327 1952

55 Lathem W Baker K and Bradley S Urinary amino acid excretion in renal disease with observations on the Fanconi syndrome Am J Med 18 249 1955

56 Robson E B and Rose G A The effect of intravenous lysine on the renal clearances of cystine arginine and ornithine in normal subjects in patients with cystinuria and Fanconi syndrome and in their relatives Clin Sc 16 5 1957

57 Beyer K H Wright L D Skeggs H R Russo *H F and Shaver G A* Renal clearance of essential amino acids their competition for reab orption by the renal tubules Am J Physiol 151 202 1947

58 Pitts R F A comparison of the renal reabsorptive processes for several amino acids Am J Physiol 140 535 1944

reached levels several fold greater than the concentration in glomerular filtrate at $T_m$. The splay in the glucose titration curve for the whole kidney as well as for the individual nephron could be explained on this basis

The nature of the hypothetical cellular carrier if indeed one exists has not been elucidated It has been postulated that the initial step in tran port is phosphorylation of glucose mediated by hexokinase and adenosine triphosphate and subsequent dephosphorylation through the action of a phosphatase [16 17] The glucose 6 phosphate formed in the first reaction is assumed not to diffuse out of the cell and the removal of *free* glucose by phosphorylation would then favor the inward movement of glucose in the direction of the concentration gradient Hexokinase non specific alkaline phosphatase and the specific glucose 6 phosphatase are found in proximal tubular cells However the weight of evidence is heavily against a phosphorylated sugar as an intermediate in the transport process [10] When phlorizin was administered to animals to produce almost complete block of glucose reabsorption no inhibition of alkaline phosphatase in the proximal convoluted tubules shown histochemically could be found [18] Phlorizin in doses which produced profound glycosuria did not alter the time curve of disappearance of injected $P^{32}$-labeled inorganic phosphate from the plasma of cats [19] These data of Dratz and Handler [19] indicated that in the presence of phlorizin, normal or even accelerated synthesis of glucose esters is seen The authors con cluded that if a highly active fraction of either glucose 1 phosphate or glucose 6-phosphate mediates renal tubular transport of glucose then this must be present in amounts too minute to be detected by their technique In addition there are two clinical observations which fail to support the unique role of either alkaline or glucose 6-phosphatase in the transport reactions Cori noted the absence of glycosuria in patients with glycogen storage disease in whom the specific glucose 6-phosphatase was lacking [20] Similarly normal carbohydrate metabolism has been observed in subjects with hypophosphatasia in whom alkaline phosphatase activity is decreased or absent [21]

Additional evidence against the phosphorylation hypothesis has been obtained through studies of active transport of sugars in the small in testine a situation analogous to that in the renal tubule The experiments of Sols on the sub trate specificity of intestinal hexokinase demonstrate clearly that this enzyme cannot directly participate in active transport [22] He found that several hexoses actively transported by the intestine were not pho phorylated by intestinal homogenates More directly it has been shown that certain hexoses lacking a hydroxyl group at carbon atoms 1 or 6 can still be transported by intestinal sacs in vitro against a concentration gradient [23] Further examination of the substrate peci ficity of this reaction has shown that those compounds are tran ported

transport of glucose This subject has been recently reviewed by Taggart [10]

The application of micropuncture techniques has demonstrated in various Amphibia and mammals that glucose is present in the glomerular filtrate in the same concentration as in the plasma water [11 12] In the proximal convoluted tubule sugar is reabsorbed against an increasing concentration gradient, so that by the mid point of this tubule little glucose remains in the lumen The reabsorptive process which is blocked by the glycoside phlorizin, is not restricted to any particular segment of the proximal tubule, but it is not demonstrable in the distal tubule In the presence of phlorizin the concentration of reducing substance in the tubular urine may increase to as high as three times that of plasma as water is reabsorbed In phlorizinized *Necturus*, glucose can diffuse back toward the plasma if the concentration in the tubular lumen is significantly greater than in plasma

It has been shown in the dog by Shannon [13] and in man by Smith [14] that as the concentration of glucose in the blood is progressively increased the amount reabsorbed ($T$) by the tubules approaches a maximum ($T_m$) Shannon assumed that as the load of glucose presented to the tubules increases progressive saturation of the reabsorptive mechanism occurs until a maximum level is reached and the excess is excreted in the urine He postulated that the sequence of reactions in glucose transfer involves (1) a reversible combination of the solute with a hypothetical cellular element present in constant but limiting amounts and (2) decomposition of this complex by a reaction which is first-order in type and rate limiting for the whole process

Although Shannon s data fitted well with his hypothesis clearance data obtained in man show variable deviations, or splay from the ideal curve [14] The explanation has been offered that even in the normal kidney with loads less than the maximal average the transport in some individual nephrons will be less than in others [15] Since the capacity of some tubules for reabsorption will be exceeded at relatively low levels glucose would be excreted from these nephrons before the average maximal load is reached It has been further assumed that the titration curves for both kidneys would deviate from the "theoretical" in proportion to the degree of heterogeneity in the nephron population

It has however been pointed out by Mudge [15] and Berliner [49] that the assumptions on which this explanation is based can be only an approximation, i e , that each nephron excretes no glucose until completely saturated There are no experimental data which conclusively prove this On the contrary if Michaelis Menten kinetics are applicable to the process of glucose transport across a tubule cell it is apparent that maximal rates would not be attained until concentration of the substrate

## NATURE OF THE TUBULAR ABNORMALITY IN RENAL GLYCOSURIA

It is not possible to localize the fundamental defect in renal glycosuria With the exception of two cases published in 1935 and 1939 there is no recent report of pathologic anatomic findings in this disease Marble [1] has pointed out that in the earlier publication of Grote and Heilmann [36] the patient probably had true diabetes mellitus The other case reported by Monasterio [37] was evidently one of true renal glycosuria with a similar disease in a sibling and diabetes mellitus in one parent Histologic examination of a biopsy specimen of one kidney showed in many places marked flattening of tubular epithelium this was occasionally so striking that the epithelium resembled endothelium The author was careful to note that the relationship of this change to the functional abnormality was not certain

There have been several studies of patients with renal glycosuria using clearance techniques [38-42] The glomerular filtration rate [38-40] secretion of p aminohippuric acid [38] reabsorption of phosphate [43] and amino acids [15] have been reported normal in these patients There is no evidence of generalized progressive renal failure

Data on glucose reabsorption as reported by several groups of investigators are summarized in Table 38 1 It was originally propo ed by Reubi [38] and Bradley [40] that the patients seemed to fall within two separate groups those with normal glucose $T_m$ and those with low glucose $T_m$ Reubi concluded that in those patients with low glucose $T_m$ a familial tendency was evident Bradley thought the reverse was true Taggart [44] in reviewing the available literature including the data collected by Bradley Reubi and Lambert concluded that the cases did not appear to fall into two distinct categories according to the glucose $T_m$ but were evenly distributed from the lowest to the highest values This is evident as shown in Table 38 1

Data obtained with the titration method of Smith [14] are of considerable interest Use of this technique in the patients with low glucose $T_m$ has led to the conclusion that the defect in glucose reabsorption is diffuse involving most if not all nephrons In the other individuals with renal glycosuria in whom the gluco e $T_m$ was within the normal range both Reubi and Bradley commented upon the exaggerated splay in the titration curves They attributed this to a heterogeneity among nephrons whether on a functional or anatomic basis Reubi further suggested that in these individuals there were actually two distinct groups of nephrons However as previou ly mentioned configuration of the titration curve may be a function of the kinetics of the tubular tran fer reactions rather than an expression of heterogeneity among nephrons For example a

which possess a D pyranose ring, a methyl or substituted methyl group at carbon 5 of this structure, and a hydroxyl group in the glucose configuration at carbon 2 [24] a structural specificity differing distinctly from that of mammalian hexokinase [25] Reactions which involve removal or transfer of oxygen at carbon 2 of the sugar molecule or which require the presence of carbon bound hydrogen at the same position have further been excluded [26] In the kidney it has also been shown by Chinard who infused glucose 1 $C^{14}$ into the renal artery of anesthetized dogs, that the sugar is transported without breakdown and resynthesis of the six carbon chain [27]

Some comment concerning the inhibitory effect on renal sugar transport of the glycoside phlorizin is pertinent since many of the hypotheses discussed above assume that the action of this compound is highly specific Phlorizin has been shown to inhibit several nucleotide requiring enzymes such as phosphorylase [28] Ehrlich ascites tumor hexokinase [29] mutarotase [55], and aerobic phosphorylation in homogenates of guinea pig kidney cortex [30] In addition, it inhibits the entrance of sugars into erythrocytes [31] and Ehrlich ascites tumor cells [32] which do not possess a mechanism for the accumulation of these substances against a concentration gradient It has recently been shown that slices of rabbit and rat kidney cortex can accumulate D galactose 1 $C^{14}$ a sugar reabsorbed by mammalian kidney tubules, against an apparent concentration gradient [33] In the absence of oxygen or in the presence of dinitro-ortho cresol (an inhibitor of oxidative phosphorylation) the amount of galactose entering the cells is limited to that expected from diffusion equilibrium The extent of accumulation in a given time period is a function of the external galactose concentration and shows a maximal rate Glucose competitively inhibits this accumulation Phlorizin in concentrations previously shown to be active in vivo inhibits the entrance of galactose into the tissue not only under conditions where accumulation can occur but also when accumulation has been inhibited by dinitro ortho cresol In the light of the results obtained with this in vitro model oth r experiments which minimize the role of diffusion of glucose from the tubular lumen in the presence of phlorizin must be reevaluated Diffusion becomes evident only when the process of active transport is blocked [34]

In summary glucose is present in the glomerular filtrate in concentration equal to that in the plasma water It is reabsorbed in the proximal convoluted tubules against an ever increasing concentration gradient Reabsorption of the sugar is mediated by energy requiring processes has a restricted specificity and is susceptible to inhibition by phlorizin, which in some manner restricts penetration of sugar into the cell A maximal rate of transport is observed both in vitro and in vivo The nature of the transport mechanism remains obscure and to date no chemical reaction involving the transported sugar has been demonstrated

of the tubular membrane towards the diffusion of glucose could also result in a change in the shape of the titration curve in the same direction

One can therefore only speculate concerning the tubular abnormality in renal glycosuria It is possible that no one explanation will suffice to account for the mechanism of the defect in all cases

## GENETICS

The abnormality in renal glycosuria is probably *inherited as a Mendelian dominant characteristic* [2 7 8] Further examination of members of affected families using more refined techniques of examination (such as Smith s titration method [9]) is indicated before this can be established with certainty

The most complete study of the heredity of renal glycosuria was published in 1927 [47] Hjarne was able to obtain information on 141 out of 199 individuals in three generations who had common ancestors in the eighteenth century Glycosuria with normal blood sugar levels was found in 18 on unlimited carbohydrate intake and an additional 6 persons had glycosuria during the course of a glucose tolerance test only Hjarne concluded that the defect was inherited as a dominant characteristic in view of the following

1 *Glycosuria occurred in male as well as female members of the family*

2 Where neither parent had glycosuria none of the offspring had the abnormality

3 Where either of the parents had glycosuria some of the children generally had it

Although true diabetes mellitus was present in 7 members of the family studied by Hjarne the mode of inheritance of this defect was not similar to that of renal glycosuria Similar conclusions regarding inheritance of renal glycosuria were reached by Schnell [7] and supported by the report of Brown and Poleshuck [48] So far the heterozygote has not been differentiated from the homozygote—although Froesch et al [46] have suggested from the evaluation of titration curves in three brothers with the defect the possibility that the defect may be expressed in the heterozygote but more distinctly in the homozygote

## SUMMARY

1 Renal glycosuria is an abnormality in which glucose is excreted in the urine at normal concentrations of blood glucose The condition probably inherited as a *Mendelian dominant trait* is a benign one unassociated with any clinical abnormality

2 It is still not certain whether several different abnormalities are included under the name *renal glycosuria* Separate entities could depend

TABLE 38-1 DATA ON GLUCOSE REABSORPTION

| No | Case | Sex | Inulin clearance ml/min | Glucose $T_m$ mg/min | Ref |
|---|---|---|---|---|---|
| 1 | M L | M | 111 | 204 | [38] |
| 2 | H E | M | 152 | 220 | [38] |
| 3 | P S | M | 134 | 219 | [38] |
| 4 | F L | M | 126 | 144 | [38] |
| 5 | R S | M | 116 | 153 | [38] |
| 6 | I K | M | 99 | 151 | [38] |
| 7 | E H | M | 108 | 275 | [38] |
| 8 | K B | F | 109 | 304 | [38] |
| 9 | H M | M | 84 | 53 | [38] |
| 10 | Sci | F | 121 | 104 | [39] |
| 11 | Def | F | 105 | 93 | [39] |
| 12 | Tim | M | 144 | 131 | [39] |
| 13 | H P | | 87 | 1 8* | [40] |
| 14 | A G | | 119 | 169 | [40] |
| 15 | O L | | 133 | 204 | [40] |
| 16 | R B | | 111 | 294 | [40] |
| 17 | D A | | 136* | 325 | [40] |
| 18 | | F | 90 | 145 | [40] |
| 19 | | F | 134† 102‡ | 116 225 | [41] |
| 20 | Ch N | M | 98 | 200 | [46] |
| 21 | R N | M | 97 | 141 | [46] |
| 22 | F N | M | 114 | 141 | [46] |
| 23 | W H | M | 98 | 139 | [46] |
| Normal (± s d ) | | | 130 ± 15 | 350 ± 50 | [44] |

* Values corrected to 1 73 $m^2$ body surface
† Values in late pregnancy
‡ Values 6 months following delivery In this case studied early in pregnancy no glucose $T_m$ was observed with plasma glucose concentrations of 888 mg/100 ml $T$ glucose was 810 mg/min

decrease in the affinity of the substrate (glucose) for the transfer system could result in a shift of the curve to the right without altering $T_m$ [1] Thus the point at which the ratio $T/T_m$ reaches 0 5 would occur at higher plasma concentrations of glucose Changes in the permeability characteristics

[1] The amount of glucose reabsorbed per minute ($T$) is calculated by subtracting the amount appearing in the urine per minute from the filtered load (plasma concentration × glomerular filtration rate in milliliters per minute) It is apparent that $T$ glucose represents a mean rate of tubular reabsorption since the amount reabsorbed decreases progressively along the proximal convoluted tubule Until the maximal rate of reabsorption ($T_m$) is reached it is usually considered that $T$ is a function of load However since the glomerular filtration rate does not significantly change in any one study as plasma glucose concentration is increased then $T$ may be considered a function of plasma glucose concentration Although $T$ does not represent true initial velocity for the reabsorption process if the system follows Michaelis Menten kinetics then an approximate apparent $K_m$ would be the concentration of plasma glucose when $T/T_m = 0.5$ ($V/V_{max} = 0.5$) This problem has been discussed by Wilbrandt [45]

2 Sols A The hexokinase activity of the intestinal mucosa Biochim et biophys. acta 19 144 1956
23 Crane R K and Krane S M On the mechanism of the intestinal absorption of sugars Biochim et biophys acta 20 568 1956
24 Wilson T H and Crane R K The specificity of sugar tran port by the hamster intestine Biochim et biophys acta 29 30 1958
25 Sols A and Crane R K Substrate specificity of brain hexokinase J Biol Chem 210 581 1954
6 Crane R K and Krane S M Studies on the mechan sm of the intestinal active transport of sugars Biochim et biophys acta 31 33 1959
27 Chinard F P Taylor W R Nolan I F and Enns T Renal handling of glucose in dogs Am J Physiol 196 535 1959
8 Cori C F Cori G T and Green A A Crystalline muscle phosphorylase III Kinetics J Biol Chem 151 39 1943
29 Crane R K Unpublished observations
30 Lotspeich W D and Keller D M A study of some effects of phlorizin on the metabolism of kidney tissue in vitro J Biol Chem 222 843 1956
31 Le Fevre I G Evidence of active transfer of certain non-electrolytes across human red cell membrane J Gen Physiol 31 05 1948
32 Crane R K Field R A and Cori C F Studies of tissue permeability I The penetration of sugars into the Ehrlich a c tes tumor cells J Biol Chem 224 649 1957
33 Krane S M and Crane R K The accumulation of D-galactose against a con centration gradient by slices of rabbit kidney cortex J Biol Chem 234 211 1959
34 Lotspeich W D and Woronkow S Some quantitative studies on phlorizin inhibition of glucose tran port in the kidney Am J Physiol 195 331 1958
35 Keston A S Occurrence of mutarotase in animals its proposed relationship to transport and reab orption of sugars and insulin Science 120 355 1954
36 Grote L R and Heilmann P Zentralbl allg Path 64 65 1935 (in A Marble [4])
37 Monasterio G Histologischer und physio pathologischer Beitrag zur Pathogenese der Diabetes renalis Klin Wchnschr 18 538 1939
38 Reubi F C Glucose titration in renal glycosuria in *Ciba Foundation Symposium on the Kidney* Little Brown Boston 1954
39 Lambert P P A study of the mechanism by which tubular damage changes the renal threshold in *Ciba Foundation Symposium on the Kidney* Little Brown Boston 1954
40 Bradley S E Bradley G P Tyson C J Curry J J and Blake W C Renal function in renal diseases Am J Med 9 766 1950
41 Robertson J A and Gray C H Renal function tests in renal glycosuria including observations during pregnancy Lancet 2 15 1950
42 Nielsen A L On the mechanism of glycosuria I Acta med scandinav 130 219 1948
43 Govaerts P and Lambert P P Pathogenie du diabete renal Acta clin belg 4 1 1949
44 Taggart J V In Combined clinic on disorders of renal tubular function Am J Med 20 448 1956
45 Wilbrandt W Secretion and tran port of non-electrolytes in *Symposia of the Soc ty f Experimental Biology* vol 7 Academic Press Inc New York 1954
46 Froesch E R Winegrad A I and Renold A L Die tubulare Nierenfunktion bei verschiedenen Formen des renalen Diabetes Mellitus Helvet med acta 24 518 1957
47 Hjelt V A study of orthoglycaemic glycosuria with particular reference to its heredit ability Acta med scandinav 67 47 1..
48 Brown M S and Pole Luck R Familial renal glycosuria J Lab Clin Med 20 605 1935
49 Berliner R Discussion of paper by F C Reubi [38]

upon a reduction of renal threshold on the one hand or upon a decrease in the maximal capacity of the renal tubule to reabsorb glucose on the other

3 The majority of cases studied show a decrease in $T_m$ glucose and a variable degree of "splay" of the titration curve It remains to be determined whether the abnormality is diffuse in all cases or the result of a heterogeneous population of nephrons in some cases

4 The fundamental mechanism of the renal tubular transport of glucose remains obscure, until more is known about this process the abnormality in renal glycosuria cannot be further defined

## BIBLIOGRAPHY


1 Marble A Non-diabetic melituria in *The Treatment of Diabetes Mellitus* by E I Joslin H F Root P White and A Marble Lea & Febiger Philadelphia 1952

2 Lawrence R D Symptomless glycosurias differentiation by sugar tolerance tests M Clin North America 31 289 1947

3 Marble A Renal glycosuria Am J M Sc 183 811 1932

4 Marble A *The diagnosis of the less common melitunas including pentosuria* and fructo uria M Clin North America 31 289 1947

5 Ackerman I P Fajans S S and Conn J W The development of diabetes mellitus in patients with nondiabetic glycosuria Clin Res Proc 6 251 1958

6 Horowitz L and Schwarzer S Renal glycosuria occurrence in two siblings and a review of the literature J Pediat 47 639 1955

7 Schnell A Das Wesen der essentiellen renal Glykosurie und ihre Beziehung zum Diabetes Mellitus unter besonderer Berucksichtigung der Erblichkeitsfrange Acta med scandinav 92 153 1937

8 Houston J C and Merrivale W H H Renal glycosuria in a family Guy's Hosp Rep 98 233 1949

9 Smith H W *Lectures on the kidney* University of Kansas Lawrence 1943

10 Taggart J V Mechanisms of renal tubular transport Am J Med 24 774 1958

11 Walker A M and Hudson C L The reabsorption of glucose from the renal tubules in amphibia and the action of phlorizin upon it Am J Physiol 118 150 1937

12 Walker A M Bott P A Oliver J and MacDowell M C The collection and analysis of fluid from single nephrons of the mammalian kidney Am J Physiol 134 580 1941

13 Shannon J A and Fisher S The renal tubular reabsorption of glucose in the normal dog Am J Physiol 122 765 1938

14 Smith H W *The Kidney Structure and Function in Health and Disease* Oxford University Press New York 1951

15 Mudge G H Clinical patterns of tubular dysfunction Am J Med 24 785 1958

16 Lundsgaard E Die Wirkung von Phlorrhizin auf die Glucoseresorption Biochem Ztschr 264 221 1933

17 Kalckar H Phosphorylation in kidney tissue Enzymologia 2 47 1937

18 Kritzler R A and Gutman A B Alkaline phosphatase activity of the proximal convoluted tubules and the mechanism of phlorizin glycosuria Am J Physiol 134 94 1941

19 Dratz A F and Handler P Renal phosphate and carbohydrate metabolism studied with the aid of radio phosphorus J Biol Chem 197 419 1952

20 Cori G T Glycogen structure and enzyme deficiencies in glycogen storage disease Harvey Lect 48 145 1954

21 Fraser D Hypophosphatasia Am J Med 22 730 1957

detailed here Many attempts have been made to define the nature of the underlying tubular defect and the resulting pathogenesis of the changes in blood and urine From these studies has come general agreement on the clinical features and the over all pathogenesis of the disorder, but a more precise definition of the disease at the cellular or enzymatic level remains to be obtained It is the purpose of this chapter to review current concepts of the nature of the tubular disorder in renal tubular acidosis and to discuss how this disorder may relate to the clinical manifestations of the disease

## CLINICAL AND PATHOLOGIC MANIFESTATIONS

Since the major attention of this chapter is to be directed toward pathogenesis the clinical syndrome of renal tubular acidosis will be only briefly summarized Excellent descriptions of typical case histories are available elsewhere [8–10] In general the syndrome has been described✓ in two groups of patients—in infancy or in older children and adults In infancy the clinical picture is quite nonspecific and is often described under the all inclusive term failure to thrive There may be anorexia nausea vomiting polyuria dehydration hypercalcemia and fever Nephrocalcinosis visible to x ray examination is rare If the patient is treated adequately the ultimate prognosis is excellent There is complete✓ remission in the majority of cases [11] This syndrome in infancy has not been so well studied as in the adult It is not entirely clear that these two groups of patients have the same disorder

In older children and adults the constant acidosis (retention of $H^+$) may be associated with nonspecific complaints of anorexia lethargy fatigue and failure of normal growth The most serious complications relate to the loss of cations from the body e pecially calcium and potassium Depletion of calcium may be associated with rickets in childhood or osteomalacia in adults [9] During the excessive urinary loss of calcium local renal complications such as nephrocalcinosis and nephrolithiasis frequently occur The excessive urinary loss of potassium may lead to recurrent attacks of weakness or even flaccid paralysis The patient may have polyuria with impairment of urine concentration The extracellular fluid pattern is that of a metabolic acidosis associated with hyperchloremia In addition studies may reveal hypokalemia or the chemical pattern in the serum of rickets or osteomalacia i e low normal to low calcium low pho phorus and high alkaline pho phatase concentrations In Fig 39-1 is summarized the incidence of certain of the clinical features of renal tubular acidosis as found in 28 patients [7–10 12–19] The average age at the time of diagnosis in this series was 29 although symptoms often preceded diagnosis by a number of years Patients are listed as showing $K^+$ depletion only if there were symptoms which

# Chapter 39

# Renal Tubular Acidosis

*Lloyd H Smith Jr*

Renal tubular acidosis is a descriptive term for a rare sometimes familial disorder of the renal tubule characterized by an inability to excrete a urine of normal acidity This primary defect leads to a continuing hyperchloremic acidosis which is often associated with one or more secondary complications such as hypercalcinuria with nephrolithiasis and nephrocalcinosis rickets or osteomalacia and severe potassium depletion

Most of the disorders of renal tubular function which have been described as single entities are occasionally found as components of multiple tubular defects This is true for renal tubular acidosis which may be one of the spectrum of abnormalities of the Fanconi syndrome [1] (cf Chap 37) The syndrome of renal tubular acidosis may be clo ely simulated by the chronic administration of potent carbonic anhydrase inhibitors a fact of considerable theoretical importance in speculations about its pathogenesis Finally the extracellular fluid pattern of chronic hyperchloremic acidosis may be found following bilateral ureterosigmoid transplantations [2] or during the continued ingestion of large amounts of ammonium chloride A similar pattern has been described in a group of patients with chronic pyelonephritis [3]

First descriptions of renal tubular acidosis were those of Lightwood in 1935 [4] and of Butler, Wilson and Farber in 1936 [5] The syndrome was subsequently studied extensively by Albright and his collaborators with clarification of the major features of the over all physiologic defect in renal function [6] From these studies plausible explanations for most of the systemic complications were offered and a rational therapeutic regimen was proposed Over the last two decades the disorder of renal tubular acidosis has been recognized with increasing frequency and described under at least 14 separate terminologies [7] which will not be

tion reactions in intermediary metabolism. From a quantitative standpoint the lung with its feed back control adjustment for excreting this volatile anhydride of carbonic acid is the organ par excellence of acid base balance in that it can excrete a hundredfold more acid than the kidney and is capable of minute by minute adjustment of body pH. A second normal stress on the maintenance of pH is the metabolic liberation of certain acidifying anions of dietary origin particularly phosphate and sulfate. On an average dietary intake these represent approximately 50 to 100 mEq excess acid per day. It is in the controlled excretion of this fixed acid[1] load that the kidney primarily serves to rid the body of excess hydrogen ions. The mechanisms by which the kidney serves this function can be arbitrarily divided into several categories. It will be apparent that these are in fact closely related phenomena. (1) excretion of sulfate and phosphate (2) conservation of filtered bicarbonate (3) tubular secretion of hydrogen ion (4) tubular synthesis and excretion of ammonia.

*Excretion of Sulfate and Phosphate*

From many clearance studies it has been established that phosphate and sulfate are filtered by the glomerulus and partially reabsorbed by the tubule. Retention of these anions sufficient to produce acidosis is invariably associated with marked reduction of glomerular filtration. This is the type of renal acidosis found in most patients with uremia. If the retention of these anions were completely balanced by a corresponding retention of cations (largely the sodium ion) no acidosis would result. In the cross purpose of maintaining the constancy of intracellular and extracellular osmolarity this general increase of cation concentration is not allowable. These anions therefore displace the anions of weaker acids especially bicarbonate. Unless there is a corresponding reduction of carbonic acid mediated by the respiratory center and lung acidosis is the inevitable result.

*Conservation of Filtered Bicarbonate*

The kidney is primarily concerned with the conservation of the corresponding buffer base (largely sodium) rather than the bicarbonate ion per se which is almost limitlessly available from metabolic carbon dioxide. It is evident that the maintenance of acid base balance is endangered by the profligacy of a glomerular filtration of approximately 4.5 moles bicarbonate per day approximately fifteen times that normally present

[1] In the discussion to follow the terms *acid* and *base* are employed in the conventional clinical usage which is incorrect as chemical terminology (2, 3). The terms imply the physiologic effects of certain anions and cations respectively rather than their properties as proton donors or acceptors. Switching to more correct usage here would probably result in loss of clarity.

seemed clearly related to potassium loss Other patients exhibited varying degrees of hypokalemia

The few autopsy studies of the kidney in renal tubular acidosis have failed to reveal a constant pathologic picture In general the glomeruli have been uninvolved Interstitial calcification, evidence of pyelonephritis and vacuolization of tubular cells have been reported [6] No abnormality comparable to the "swan neck" deformity of the tubule in the Fanconi syndrome has been described [20] The tubular changes may be in part attributable to chronic potassium depletion [21]

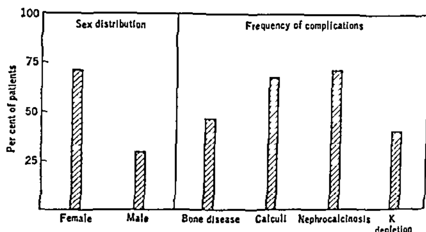



Fig 39-1 The incidence of certain of the clinical features of renal tubular acidosis as found in 28 patients from the literature [ -10 12 1J]

## PATHOGENESIS

Renal tubular acidosis is associated with a deficient acidification of the urine despite a continuing systemic acidosis When the acidification mechanism *is placed under additional stress by the use of acid or acid forming* salts there is little or no further decrease in urine pH Response of the kidney to systemic acidosis is therefore maximal but inadequate Clearly the logical point of departure in investigating this syndrome has *been the normal regulatory role of the kidney* in acid base balance in an attempt to determine in what manner this normal pattern is deranged

### ROLE OF THE KIDNEY IN ACID BASE BALANCE

To attempt a general review of acid base balance would be presumptuous in the present context The following summary will relate only to the mechanisms by which the kidney contributes to the *physiologic control of the hydrogen ion over the tenfold range compatible with life*

In man there is normally a constant tendency toward acidosis largely because of the liberation of carbon dioxide by certain specific decarboxyla

tion reactions in intermediary metabolism From a quantitative standpoint the lung with its feed back control adjustment for excreting this volatile anhydride of carbonic acid is the organ par excellence of acid base balance in that it can excrete a hundredfold more acid than the kidney and is capable of minute by minute adjustment of body pH A second normal stress on the maintenance of pH is the metabolic liberation of certain acidifying anions of dietary origin particularly phosphate and sulfate On an average dietary intake these represent approximately 50 to 100 mEq excess acid per day It is in the controlled excretion of this fixed acid [1] load that the kidney primarily serves to rid the body of excess hydrogen ions The mechanisms by which the kidney serves this function can be arbitrarily divided into several categories It will be apparent that these are in fact closely related phenomena (1) excretion of sulfate and phosphate (2) conservation of filtered bicarbonate (3) tubular secretion of hydrogen ions (4) tubular synthesis and excretion of ammonia

*Excretion of Sulfate and Phosphate*

From many clearance studies it has been established that phosphate and sulfate are filtered by the glomerulus and partially reabsorbed by the tubule Retention of these anions sufficient to produce acidosis is invariably associated with marked reduction of glomerular filtration This is the type of renal acidosis found in most patients with uremia If the retention of these anions were completely balanced by a corresponding retention of cations (largely the sodium ion) no acidosis would result In the cross purpose of maintaining the constancy of intracellular and extracellular osmolarity this general increase of cation concentration is ✓ not allowable These anions therefore displace the anions of weaker acids especially bicarbonate Unless there is a corresponding reduction of carbonic acid mediated by the respiratory center and lung acidosis is the inevitable result

*Conservation of Filtered Bicarbonate*

The kidney is primarily concerned with the conservation of the corresponding buffer base (largely sodium) rather than the bicarbonate ion per se which is almost limitlessly available from metabolic carbon dioxide It is evident that the maintenance of acid base balance is endangered by the profligacy of a glomerular filtration of approximately 4.5 moles ✓ bicarbonate per day approximately fifteen times that normally present

[1] In the discussion to follow the terms *acid* and *base* are employed in the conventional clinical usage which is incorrect as chemical terminology [*22 23*] The terms imply the physiologic effects of certain anions and cations respectively rather than their properties as proton donors or acceptors Switching to more correct usage here would probably result in loss of clarity

in the entire extracellular fluid This must be largely reabsorbed by tubular action Evidence from several sources has indicated that glomerular filtrate is reabsorbed in the proximal segment of the tubule with little change in ionic concentration or pH [24] It follows that five sixths of bicarbonate must be similarly reabsorbed here It should be noted that some acidification has recently been demonstrated by micropuncture in the proximal tubule of the rat [25] Acidification of the urine which occurs in the distal tubular segment, is associated with the reabsorption of most of the remaining bicarbonate in proportion to the final hydrogen ion concentration attained It was previously postulated that the reabsorption of bicarbonate by these two segments occurred through different mechanisms—proximal reabsorption representing an active transport mechanism distal reabsorption resulting from an ion-exchange reaction of $H^+$ for $Na^+$ with back diffusion of the released carbon dioxide [26] Recently the use of more powerful carbonic anhydrase inhibitors has indicated that the reabsorption of bicarbonate is related to ion exchange at both sites [27] the essential difference lying in the ability of the distal tubular cell to concentrate $H^+$ against a positive gradient The reabsorption of bicarbonate is therefore intimately connected with the tubular secretion of $H^+$, which will be discussed below Barring marked shifts in urine $P_{CO_2}$ the urine bicarbonate is a direct function of urine pH as signified by the Henderson Hasselbalch equation "Bicarbonate wasting" therefore is roughly synonymous with defective acidification of the urine, as seen in renal tubular acidosis and in some patients with the more usual forms of chronic nephritis [28]

*Tubular Secretion of Hydrogen Ions*

Hydrogen ion is concentrated in urine in excess of that found in plasma with the usual acid ash diet and the concentration rises still further to compensate for systemic acidosis This simple fact has long been known The mechanism by which this is effected and controlled is still under investigation Considerable information has been obtained by imaginative use of classical clearance techniques Hydrogen ion secretion is part of the general phenomenon of ion exchange across cell membranes, an area of investigation which is just beginning to be approached in vitro

For some time the assumption was made that acidification of the urine is effected by the selective tubular reabsorption of the more basic members of filtered buffer pairs—i e $Na_2HPO_4$ in preference to $NaH_2PO_4$ Hydrogen ions would therefore be progressively concentrated intraluminally It was implicit in this theory that all $H^+$ appearing in the urine has traversed the glomerular membrane This conception of urine acidification was clearly demonstrated to be untenable in the classical studies of Pitts and Alexander [29] Under their experimental conditions of acidosis and buffer infusions in dogs it was shown that $H^+$ appeared in

the urine (largely coupled with buffer as titratable acidity) in amounts considerably in excess of that which could be accounted for by glomerular filtration The suggestion was made that the tubular cells secreted $H^+$ into the lumen in exchange for $Na^+$ This exchange would allow a net loss *of protons and simultaneously retrieve* buffer base from the tubular urine The source of $H^+$ for this exchange was thought to be $H_2CO_3$ produced by the hydration of carbon dioxide and catalyzed by carbonic anhydrase The evidence in favor of such a pivotal function of carbonic anhydrase in urine acidification is indirect but impressive a source of $H^+$ within the renal tubular cells must be constantly available and capable of producing large amounts of exchangeable $H^+$ (approximately 3 *to 4 mEq* $H^+$ *per min) Carbon dioxide as the major* acid metabolically produced in the body would meet this requirement Carbonic anhydrase is present in kidney cortex in high activity [*30*] Finally a variety of chemicals known to inhibit carbonic anhydrase in vitro (notably sulfanilamide and more recently acetazoleamide) interfere quickly and specifically with the renal acidification mechanism No effects on other tubular functions have been detected [*31*]

*The mechanism which controls the cationic exchange of* $H^+$ *for* $Na^+$ across the cell membrane is not clear The exchange in the proximal tubular segment is to be contrasted with that found in the distal segment as to both major function served and concentration gradient of $H^+$ obtained As previously pointed out the proximal exchange mechanism seems to be primarily designed to retrieve buffer base in the form of bicarbonate—perhaps four fifths or five sixths of that filtered being reabsorbed *in this way Micropuncture studies indicate that there is no* change in pH in the proximal tubule i e no net concentration of $H^+$ [*32*] Pitts has discussed the theory that the primary event is an active tubular reabsorption of $Na^+$ in excess of $Cl^-$ creating the observed negative intraluminal potential [*33*] Hydrogen ions derived from carbonic acid would then simply diffuse into the lumen along an electrical gradient Since there is no net concentration of $H^+$ there is no associated diffusion of ammonia This presumed diffusion of $H^+$ seems to have no relationship to potassium secretion into the lumen If in fact proximal reabsorption of bicarbonate is wholly dependent on $H^+$ $Na^+$ exchange an impressive total of 2 5 to 3 0 mEq $H^+$ per min must be produced and transferred by the proximal tubule

The hydrogen ion–exchange mechanism in the distal tubular segment seems also to depend on the activity of carbonic anhydrase In other respects it differs markedly from the simple diffusion thought to occur in the proximal segment When maximally stimulated there is a concentration gradient of $H^+$ of about eight hundredfold across the tubule (pH 4 6 in urine 7 4 in peritubular fluid) Although the exchange allows the salvage of the final one fifth or one sixth of bicarbonate buffer base

it also exchanges with the buffer bases of other filtered buffers, especially phosphate titrating them to a more acidic form Finally the increased intraluminal $H^+$ concentration appears to be a major factor leading to the diffusion of ammonia into the tubular urine This will be discussed in more detail below The nature of the active transport mechanism which can produce an eight hundred fold gradient of $H^+$ across the inner cell membrane of the distal tubule has not been elucidated Physiologic studies have firmly established, however, that the secretion of potassium into the distal tubular urine in exchange for sodium is competitively associated with the $H^+$ transport mechanism increased $K^+$ secretion is produced by decreased $H^+$ secretion and vice versa [27] The limiting factor in $H^+$ exchange in the distal tubule is that of a maximal concentration gradient (pH) When phosphate is infused during experimental acidosis and appears in the distal tubule to buffer the secreted $H^+$ and thus to prevent the maximal pH gradient from being reached, the rate of $H^+$ secretion increases markedly [29] The maximal rate of $H^+$ secretion by this mechanism has not yet been determined

The fate of $H^+$ secreted or diffusing into the tubular lumen depends wholly on the composition of the tubular urine at that point The major products formed are $H_2O$ from the decomposition of carbonic acid the more acidic of buffer anionic pairs (especially $H_2PO_4^-$), and the ammonium ion from the capture of $H^+$ by diffused $NH_3$

*Tubular Synthesis and Secretion of Ammonia*

The central problem which the kidney faces in acid base balance is that of preserving buffer base endangered by glomerular filtration Buffer base is represented by metallic cations ($Na^+$ $K^+$, $Mg^{++}$, $Ca^{++}$) ingested as such and therefore of limited availability The hydrogen ion is a cation which is metabolically produced and is therefore available for exchange reactions in the kidney to preserve buffer base as described above Unfortunately $H^+$ exchange is limited by the attending fall in pH Ammonium is a second metabolically derived base which in effect serves to enhance the $H^+$ exchange mechanism by neutralizing intraluminal $H^+$ The gradient is thereby maintained at less than the eight hundred fold maximum and $H^+$ secretion can continue

The source of urinary ammonia was clearly shown to be the amide group of glutamine and to a lesser extent the amino groups of other amino acids in classical studies by Van Slyke and his colleagues [34] This has been widely confirmed Data from many sources have indicated that three factors determine the amount of ammonia found in the urine the availability of ammonia precursors the cellular levels of enzymatic activities productive of $NH_3$ and the pH of the urine [35] The influence of ammonia precursors is indicated by the increase of urinary ammonia found during the experimental infusion of glutamine or of a number of

other amino acids [36 37] This presumably reflects a rise of intracellular ammonia concentration produced by increased substrate availability at a given enzyme concentration Changes in substrate availability have not been shown to be of significance under other than experimental conditions With continued acido is there is a rise in urinary ammonia at a given pH In the past this rise has been assumed to be due to an adaptive

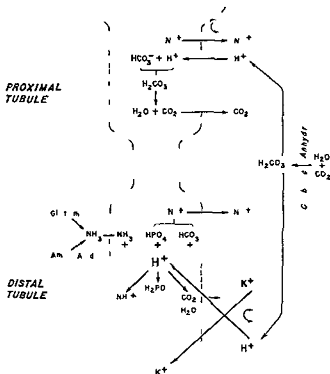



Fig 39-2 Diagram to illustrate the function of the renal tubule in regulation of acid base balance

increase of enzymatic activity within the distal tubular cell Such an adaptive increase of glutaminase in the presence of experimental acidosis has been directly demonstrated in the rat [38] Recent studies in the dog however have failed to demonstrate any adaptive increase of the enzymes known to be associated with *ammonia production* [39] *Indirect* evidence from the stimulatory effect of various ammonia precursors during chronic ammonium chloride ingestion in man suggests enzymatic *adaptation* [37] The *mechanism* by which this isohydric increase of ammonia production is brought about in man remains uncertain

Urinary ammonia is generally found to be inversely proportional to

urine pH, at any particular stage of adaptation to acidosis Evidence has been presented that ammonia, formed within the cell passes into the tubule by free diffusion down a concentration gradient [35] This is but one example of the nonionic diffusion of weak bases and acids across the renal tubule [40] In alkaline urine equilibrium is attained so that the amount of ammonia passing into the urine is limited In an acid urine ammonium ion is formed The removal of free ammonia permits diffusion to continue Seemingly the intraluminal reaction of $H^+$ and $NH_3$ serves to further the excretion of each by maintaining the necessary concentration gradient for ion exchange and free diffusion respectively

This review of the role of the kidney in acid base balance has of necessity been abbreviated and overly didactic Figure 39 2 summarizes diagrammatically many points in the above discussion In summary the kidney must rid the body of the nonvolatile anionic residues of metabolism with the minimal expenditure of buffer base This is carried out by the substitution within the tubular lumen of metabolically derived cations ($H^+$ and $NH_4^+$) for the buffer base which has been placed in jeopardy by glomerular filtration

## NATURE OF THE PRIMARY DEFECT IN RENAL TUBULAR ACIDOSIS

Renal tubular acidosis is the result of failure of the normal renal mechanisms for the defense of acid base balance Glomerular filtration remains unimpaired unless secondary complications of nephrocalcinosis, nephrolithiasis and pyelonephritis lead to renal parenchymal damage The fixed acids' (sulfate and phosphate principally) do not accumulate in extracellular fluid They are normally excreted—but at an increased cost in buffer base Despite a continuous systemic acidosis and in the face of an administered acid load there is bicarbonate wasting and inability to secrete $H^+$ and $NH_3$ into the tubular urine In brief the glomerular component of renal acid base defense is normal the tubular component is defective

In the previous section evidence was presented that bicarbonate reabsorption proximal or distal is intimately related to the hydrogen ion-exchange mechanism for sodium In general in renal tubular acidosis there is a normal direct relationship of urine bicarbonate and urine pH there being no consistent alteration of urine $P_{co}$ These facts being so bicarbonate wasting seems not to be a primary defect but merely a reflection of an inadequacy of the tubular secretion of $H^+$

The most consistent abnormality found in renal tubular acidosis is an inability to excrete urine of sufficient acidity in the face of *systemic* acidosis Stated more precisely the renal tubule does not exchange sufficient $H^+$ to retrieve the necessary amount of buffer base This is reflected in a decreased urinary titratable acidity Normally minimal urine pH is about 4 5 in renal tubular acidosis it is usually found to be

6 0 to 6 8 From previous considerations of the $H^+$ secretion mechanism several hypotheses concerning the defective acidification could be advanced (1) the tubule is unable to produce and secrete $H^+$ sufficiently rapidly for normal function of the exchange mechanism (2) the tubule is unable to attain the usual maximal transtubular gradient of $H^+$ of 800 1 (3) there is a failure of the proximal $H^+$-exchange mechanism which would result in diminished proximal bicarbonate reabsorption and consequent flooding of a normally functioning distal $H^+$ secreting mechanism The e hypotheses will be considered here briefly

Studies of the response of the kidney to intravenous phosphate infusions have been carried out by several groups of investigators This procedure which increases the available urinary buffer (i e acceptor anion for $H^+$) has regularly resulted in a marked increase of $H^+$ secretion by the tubule in renal tubular acidosis usually with some decrease in urine pH [41 42 13] Rates of $H^+$ secretion are produced which if sustained would easily correct the systemic acidosis These studies suggest that the kidney in renal tubular acidosis is quite capable of forming and secreting $H^+$ at a rate commensurate with the amount of buffer base to be reabsorbed if this exchange can occur at a reduced $H^+$ concentration gradient On the other hand the defective tubule cannot continue to secrete $H^+$ in the face of a transtubular gradient of greater than approximately 80 1 rather than the normal maximal gradient of 800 1 In oversimplified terminology the defect in renal tubular acidosis seems to be primarily one of concentration rather than production or transport of $H^+$

The hypothesis was advanced by Latner and Burnard [41] on the basis of studies of renal tubular acidosis in children that the defect lies in the proximal reabsorption of bicarbonate and that this obscures by neutralization a normally functioning distal acidifying mechanism This hypothesis was based largely on the following two observations (1) the infusion of neutral phosphate buffer led to a marked increase of $H^+$ secretion with a considerable fall in urine pH (2) urine $P_c$ s were found to be elevated in renal tubular acidosis and were considered to reflect the excessive formation of carbonic acid from $HCO_3^-$ and $H^+$ in the distal tubule It was suggested that in some way phosphate increased the proximal reabsorption of bicarbonate and thereby unmasked a more normally functioning distal $H^+$ secretion mechanism The hypothesis of Latner and Burnard can be challenged The infusion of phosphate buffer at pH 7 4 regularly results in a fall of urine pH in normal subjects The explanation of this phenomenon has remained obscure [43] Urine $P_{CO_2}$ s have not been uniformly elevated in determinations on adult patients Finally if the proximal reabsorption of bicarbonate were the only tubular defect the infusion of bicarbonate in such patients should further tax the incompetent reabsorptive mechanism and result in an exacerbation of bicarbonate wasting This experiment has been carried out It was found that

urine pH, at any particular stage of adaptation to acidosis Evidence has been presented that ammonia formed within the cell, passes into the tubule by free diffusion down a concentration gradient [39] This is but one example of the nonionic diffusion of weak bases and acids across the renal tubule [40] In alkaline urine equilibrium is attained so that the amount of ammonia passing into the urine is limited In an acid urine ammonium ion is formed The removal of free ammonia permits diffusion to continue Seemingly the intraluminal reaction of $H^+$ and $NH_3$ serves to further the excretion of each by maintaining the necessary concentration gradient for ion exchange and free diffusion respectively

This review of the role of the kidney in acid base balance has of necessity been abbreviated and overly didactic Figure 39 2 summarizes diagrammatically many points in the above discussion In summary, the kidney must rid the body of the nonvolatile anionic residues of metabolism with the minimal expenditure of buffer base This is carried out by the substitution within the tubular lumen of metabolically derived cations ($H^+$ and $NH_4^+$) for the buffer base which has been placed in jeopardy by glomerular filtration

## NATURE OF THE PRIMARY DEFECT IN RENAL TUBULAR ACIDOSIS

Renal tubular acidosis is the result of failure of the normal renal mechanisms for the defense of acid base balance Glomerular filtration remains unimpaired unless secondary complications of nephrocalcinosis nephrolithiasis and pyelonephritis lead to renal parenchymal damage The fixed acids' (sulfate and phosphate principally) do not accumulate in extracellular fluid They are normally excreted—but at an increased cost in buffer base Despite a continuous systemic acidosis and in the face of an administered acid load there is bicarbonate wasting and inability to secrete $H^+$ and $NH_3$ into the tubular urine In brief the glomerular component of renal acid base defense is normal the tubular component is defective

In the previous section evidence was presented that bicarbonate reabsorption, proximal or distal is intimately related to the hydrogen ion-exchange mechanism for sodium In general in renal tubular acidosis there is a normal direct relationship of urine bicarbonate and urine pH, there being no consistent alteration of urine $P_{CO}$ These facts being so, bicarbonate wasting seems not to be a primary defect but merely a reflection of an inadequacy of the tubular secretion of $H^+$

The most consistent abnormality found in renal tubular acidosis is an inability to excrete urine of sufficient acidity in the face of systemic acidosis Stated more precisely the renal tubule does not exchange sufficient $H^+$ to retrieve the necessary amount of buffer base This is reflected in a decreased urinary titratable acidity Normally minimal urine pH is about 4 5 in renal tubular acidosis it is usually found to be

temic hyperchloremic acidosis Because of this fact it has been speculated that renal tubular acidosis represents a tubular deficiency of carbonic anhydrase perhaps on a genetic basis There is no direct evidence to support this hypothesis its chief attraction is that of analogy Two experimental findings moreover *provide evidence against it*

Carbonic anhydrase is a metalloprotein enzyme the only known function of which is to accelerate the attainment of equilibrium of the reaction



$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

It does not affect the equilibrium concentration of carbonic acid (and hence of its dissociation product $H^+$) but only the rate at which this equilibrium concentration is reached Previously cited evidence from phosphate infusions demonstrates that the rate of secretion of $H^+$ by the tubule in renal tubular acidosis is adequate given an acceptor anion to allow this secretion to continue without an adverse shift in the $H^+$ gradient This implies that the rate of $H^+$ production within the cell (presumably under the influence of carbonic anhydrase) is not a limiting factor in the acidification of the urine It is difficult to visualize how carbonic anhydrase could influence the ability of the tubule to secrete $H^+$ against a concentration gradient Secondly studies in several patients with renal tubular acidosis have shown a response to a carbonic anhydrase inhibitor acetazoleamide with a further rise in urine pH and bicarbonate excretion [44] Whether this is a normal response is still under investigation It suggests the presence of carbonic anhydrase and its function in producing whatever urinary acidification is attained in these patients This one finding would not exclude a quantitative deficiency of the enzyme Taken in conjunction with the evidence that the rate at which $H^+$ can be secreted under proper conditions is not limiting it suggests that renal tubular acidosis cannot be attributed to a primary deficiency of carbonic anhydrase It is unlikely that the primary defect in renal tubular acidosis can be more rigorously defined until additional information is available concerning the biochemical and biophysical mechanisms involved in normal $H^+$ exchange across the cell membrane

## PATHOGENESIS OF THE COMPLICATIONS OF RENAL TUBULAR ACIDOSIS

A brief summary of some of the clinical findings in renal tubular acidosis has already been given It remains to review whether plausible explanations for these complications of the disease can be based on a presumed single primary tubular defect in $H^+$ secretion

### *Nephrocalcinosis and Nephrolithiasis*

It has been well shown that patients with renal tubular acidosis exhibit hypercalcinuria with a tendency toward hypocalcemia [6] The administra-

infused bicarbonate was reabsorbed normally in renal tubular acidosis [42 43] A localized proximal tubular defect would therefore appear to be untenable with the data at hand

There is still disagreement as to whether there is a primary defect in ammonia excretion in renal tubular acidosis In the many measurements, urinary ammonia has almost invariably been found reduced considerably below that ordinarily found in comparable systemic acidosis without kidney disease For the urine pH attained, however the urinary ammonia in

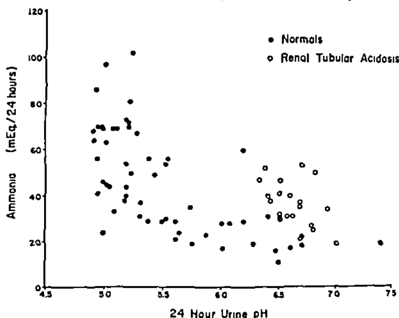



Fig 39-3 Comparison of 24 hr urine pH and ammonium content in patients with renal tubular acidosis and in control subjects [15] (*By permission of the American Journal of Medicine*)

renal tubular acidosis is normal or perhaps even increased as compared to controls with acute acidosis (Fig 39 3) In comparison with chronic experimental acidosis ammonia excretion may be somewhat diminished [37] The close relationship between $H^+$ concentration and the diffusion of nonionic $NH_3$ into the urine has been previously emphasized No determinations of glutaminase or other enzymes involved in ammonia production have been carried out on the kidney in renal tubular acidosis All in all the primary defect appears to lie in some aspect of $H^+$ secretion and concentration ammonia production being secondarily reduced because of failure to establish more favorable conditions for nonionic diffusion

Carbonic anhydrase is the only enzyme which has been implicated in the tubular formation and secretion of $H^+$ Inhibition of carbonic anhydrase causes a reduction in tubular $H^+$ secretion and may result in sys-

temic hyperchloremic acidosis Because of this fact it has been speculated that renal tubular acidosis represents a tubular deficiency of carbonic anhydrase perhaps on a genetic basis There is no direct evidence to support this hypothesis its chief attraction is that of analogy Two experimental findings moreover provide evidence against it

Carbonic anhydrase is a metalloprotein enzyme the only known function of which is to accelerate the attainment of equilibrium of the reaction



$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

It does not affect the equilibrium concentration of carbonic acid (and hence of its dissociation product $H^+$) but only the rate at which this equilibrium concentration is reached Previously cited evidence from phosphate infusions demonstrates that the rate of secretion of $H^+$ by the tubule in renal tubular acidosis is adequate given an acceptor anion to allow this secretion to continue without an adverse shift in the $H^+$ gradient This implies that the rate of $H^+$ production within the cell *(presumably under the influence of carbonic anhydrase) is not a limiting* factor in the acidification of the urine It is difficult to visualize how carbonic anhydrase could influence the ability of the tubule to secrete $H^+$ against a concentration gradient Secondly studies in several patients with renal tubular acidosis have shown a response to a carbonic anhydrase inhibitor acetazoleamide with a further rise in urine pH and bicarbonate excretion [44] Whether this is a normal response is still under investigation It suggests the presence of carbonic anhydrase and its function in producing whatever urinary acidification is attained in these patients This one finding would not exclude a quantitative deficiency of the enzyme Taken in conjunction with the evidence that the rate at which $H^+$ can be secreted under proper conditions is not limiting it suggests that renal tubular acidosis cannot be attributed to a primary deficiency of carbonic anhydrase It is unlikely that the primary defect in renal tubular acidosis can be more rigorously defined until additional information is available concerning the biochemical and biophysical mechanisms involved in normal $H^+$ exchange across the cell membrane

## PATHOGENESIS OF THE COMPLICATIONS OF RENAL TUBULAR ACIDOSIS

A brief summary of some of the clinical findings in renal tubular acidosis has already been given It remains to review whether plausible explanations for these complications of the disease can be based on a presumed single primary tubular defect in $H^+$ secretion

### *Nephrocalcinosis and Nephrolithiasis*

It has been well shown that patients with renal tubular acidosis exhibit hypercalcinuria with a tendency toward hypocalcemia [6] The administra

tion of ammonium chloride increases the hypercalcinuria, alkali therapy quickly reduces it The mechanism by which the renal tubular defect results in an increased clearance of calcium is not apparent largely because knowledge concerning the renal excretion of calcium is incomplete It has been suggested that calcium is lost as one of the 'buffer bases' because of inadequacy of urine acidification [9] On the other hand the tubular reabsorption of calcium has not been demonstrated to be dependent upon an exchange reaction with $H^+$ Hypercalcinuria occurs over long periods of time in a urine which is neutral or minimally acidic, creating favorable conditions for the precipitation of the calcium phosphate stones so frequently found It has been reported that urinary excretion of citrate is low in renal tubular acidosis, another factor which would tend to favor stone formation [40] The cause of nephrocalcinosis is not so apparent Here calcification occurs in the renal parenchyma in a disorder associated with a decreased tendency toward precipitation of bone salts in osteoid (see below) and seemingly an increased clearance (hence a decreased tubular reabsorption) of calcium It has been reported that acetazoleamide reduces urine citrate excretion and may produce renal calcinosis in the rat [46]

*Rickets or Osteomalacia*

Depending upon the age of the patient, rickets or osteomalacia may be found A major and continuing source of calcium loss in the urine has been outlined above The resulting tendency toward hypocalcemia is thought to serve as a stimulus to increased secretion of parathyroid hormone, which acts on the renal tubule to decrease the reabsorption of phosphate and directly on bone to increase its rate of destruction Dissolution of bone salts is also fostered by continued acidosis per se Bone is an important source of available buffer base Finally, there is evidence to suggest that the action of vitamin D is partially inhibited during systemic acidosis [47] The result of these interrelated events is decreased bone because of both increased destruction and delayed calcification of osteoid As a consequence bone pain or deformity may be the most striking disability associated with renal tubular acidosis

*Hypokalemia*

Balance studies on patients with renal tubular acidosis have exhibited an increased urinary loss of potassium In keeping with current concepts of potassium excretion by the kidney this probably represents tubular secretion of $K^+$ in exchange for sodium The competitive nature of the $H^+$ and $K^+$ exchange function in the distal tubule has been previously noted A diminished ability to secrete $H^+$ should therefore lead to an increased secretion of $K^+$ by the tubule On the other hand, repair of the acidosis by administering alkali further inhibits $H^+$ secretion but reduces

rather than increases urinary $K^+$ [9] In renal tubular acidosis other factors may be at play The tendency toward a negative $Na^+$ balance may lead to a secondary stimulation of aldosterone secretion which in turn would act to increase the urinary loss of potassium [48] Potassium deficiency has been well documented with hypokalemia typical electrocardiographic changes and attacks of weakness or of flaccid paralysis—all responding quickly to replacement therapy A number of patients with renal tubular acidosis have exhibited hyposthenuria and decreased responsiveness to antidiuretic hormone In the past this has been attributed to renal damage by calcification or pyelonephritis More recently the suggestion has been offered that this may relate to the clear cell nephropathy of potassium deficiency with the resulting well-documented loss of renal concentrating ability [21]

The above summary indicates that the major clinical manifestations of renal tubular acidosis relate directly or indirectly to excessive renal loss of cations This is consistent with a single renal tubular defect characterized by an inability to concentrate hydrogen ions in the urine

## FAMILIAL INCIDENCE

The inclusion of renal tubular acidosis in a compilation of inborn errors of metabolism may be legitimately criticized Of all the clinical patterns of renal tubular dysfunction it has shown least evidence of being a hereditary disorder [49] The case histories of more than 50 patients with renal tubular acidosis have been published but the familial occurrence of the syndrome has been limited to the following few reports Schreiner Smith and Kyle [7] described renal tubular acidosis with nephrocalcinosis and nephrolithiasis in two brothers The 9 year old son of one of these patients was found to have hyperchloremia rickets and nephrocalcinosis The patient s father gave a history of repeated attacks of renal colic and exhibited mild hyperchloremic acidosis Unfortunately he refused to cooperate in further studies The disorder occurred in two siblings whose case histories have been published by Foss Perry and Wood [8] The first patient was a girl of 17 with dwarfism rickets nephrocalcinosis nephrolithiasis and a history of attacks of muscle weakness suggestive of hypokalemia Her brother who was seen at age 14 was unusually short with retardation of epiphyseal development and a history suggestive of repeated attacks of renal colic Typical chemical changes of renal tubular acidosis were found in blood and urine The father of these two patients was found to have renal calculi a urine pH of 6 8 and a rather low plasma bicarbonate level The occurrence of the syndrome in twins was reported briefly by Rendle-Short [50] One of the infants died before it was established whether or not the twins were identical The other twin was said to have made a complete recovery

In a report by Cooke and Kleeman of several patients with renal tubular acidosis a footnote was added to state that nephrocalcinosis had been found in a sibling of one of their patients [51] No further details of this family have been reported Engel attributed to sulfonamide intoxication renal tubular acidosis in two siblings who subsequently made complete recoveries [52] Wilansky and Schneiderman have described a 33 year old woman with renal tubular acidosis whose father died at 65 of kidney disease with nephrocalcinosis and whose brother killed accidentally at age 20 was found to have shrunken, calcified kidneys [12] Most patients with the syndrome do not have a history to suggest a familial occurrence Similarly most studies of the immediate relatives of such patients have failed to detect a familial pattern In general these surveys have been limited to a search for overt complications of the disorder such as nephrocalcinosis nephrolithiasis, or bone disease, there has been no testing for a possible heterozygous defect such as a reduced ability to respond to an exogenous acid load The fragmentary information available does not allow resolution of the mode of inheritance of those few cases of renal tubular acidosis which have clearly been familial The presence of the disorder in two and possibly three successive generations suggests a dominant trait

The rarity of a familial pattern has led to other speculations concerning etiology It has been suggested that chronic pyelonephritis may produce in the occasional patient a selective damage to the renal acidification mechanism [6] It is true that urinary tract infection is frequently found in this group of patients who often exhibit nephrolithiasis or nephrocalcinosis or both Many patients especially those recognized in childhood have not had demonstrable infections These children often seem to make a complete recovery from the disorder contrary to the adult patients It seems more plausible to consider infection as a complication of the renal injury rather than a factor of primary etiologic importance The simulated renal tubular acidosis produced by carbonic anhydrase inhibitors has led to the theory that sulfonamides may irreversibly damage the renal tubule to impair permanently the normal acidification mechanism Most patients do not have a history of sulfonamide ingestion The widespread use of more powerful carbonic anhydrase inhibitors (such as acetazoleamide) over a period of a number of years has led to a few isolated reports of complicating nephrolithiasis [53] but no permanent interference with tubular function

In summary the etiology of renal tubular acidosis in the majority of cases remains obscure A familial occurrence has been well documented in a few incidences Other theories of etiology such as selective renal damage from pyelonephritis or carbonic anhydrase inhibitors, have failed to account for the appearance of the disease It is possible that a more careful search of the families of patients especially with provocative

tests such as the renal response to ammonium chloride would demonstrate a more consistent pattern of inheritance

## SUMMARY

1 Renal tubular acidosis is one of the more frequently encountered of the rare disorders of renal tubular function It may occur as a single defect or as one component of multiple tubular defects as in the Fanconi syndrome

2 Physiologic studies suggest that the pathogenesis lies in an inability of the tubule to attain an intraluminal hydrogen ion concentration sufficient to effect normal conservation of buffer base Whether there is an additional defect in ammonia production has not yet been established

3 The complications of this disorder relate to excessive urinary loss of cations particularly calcium and potassium Calcium loss leads to rickets or osteomalacia and local complications in the kidney such as nephrolithiasis and nephrocalcinosis Potassium loss is associated with muscle weakness and potassium deficiency nephropathy When a compensatory amount of buffer base is supplied in the diet these complications are effectively prevented

4 In a small number of patients renal tubular acidosis has been found as a familial disorder Current information does not allow resolution of the mode of inheritance but the possible occurrence in three successive generations suggests a dominant trait

5 Although studies over the past two decades have indicated the general outline of the renal defect and have led to a rational therapy the precise nature of the derangement in hydrogen ion secretion has yet to be elucidated

## BIBLIOGRAPHY


1 Wallis L. A. and Engle R L Jr The adult Fanconi Syndrome II Review of eighteen cases Am J Med 22 13 1957
2 Stamey T A The pathogenesis and implications of the electrolyte imbalance in ureterosigmoidostomy Surg Gynec & Obst 103 36 1956
3 Lathem W Hyperchloremic acidosis in chronic pyelonephritis New England J Med 258 1031 1958
4 Lightwood R Communication to British Pediatric Association Arch Dis Childhood 10 205 1935
5 Butler A M Wilson L L and Farber S Dehydration and acidosis with calcification at renal tubules J Pediat 8 489 1936
6 Albright F and Reifenstein E C Jr *Parathyroid Glands and Metabol Bone Disease* Williams & Wilkins Baltimore 1948
7 Schreiner G E Smith L H Jr and Kyle L H Renal hyperchloremic acidosis Familial occurrence of nephrocalcinosis with hyperchloremia and low serum bicarbonate Am J Med 15 122 1953
8 Foss G L Perry C B and Wood F J Y Renal tubular acidosis Quart J Med (N S ) 25 185 1956

9 Albright F Burnett C H Parson W Reifenstein E C Jr and Roos A Osteomalacia and late rickets Medicine 25 399 1946
10 Pines K L and Mudge G H Renal tubular acidosis with osteomalacia Am J Med 11, 302 1951
11 Carré I J Wood B S B and Smallwood W C Idiopathic renal acidosis in infancy Arch Dis Childhood 29 326 1954
12 Wilansky D L and Schneiderman C Renal tubular acidosis with recurrent nephrolithiasis and nephrocalcinosis New England J Med 257 399 1957
13 Reynolds T B Observations on the pathogenesis of renal tubular acidosis Am J Med 25, 503 1958
14 Albright F Consolazio W V Combs F S Sulkowitch H W and Talbott H H Metabolic studies and therapy in a case of nephrocalcinosis with rickets and dwarfism Bull Johns Hopkins Hosp 66 7 1940
15 Milne M D Stanbury S W and Thomson A E Observations on the Fanconi syndrome and renal hyperchloremic acidosis in the adult Quart J Med (N S) 21 61, 1952
16 Frick P G Rubini M E and Meroney W H Recurrent nephrolithiasis associated with an unusual tubular defect and hyperchloremic acidosis Am J Med 25 590 1958
17 Baines G H Barclay J A and Cooke W T Nephrocalcinosis associated with hyperchloremia and low plasma bicarbonate Quart J Med (N S) 14 113 1945
18 Hughes B and Gislason G J Osteonephropathy a report of two cases J Urol 55 330 1946
19 Elkinton J R Huth E J Clark J K Barker E S and Seligson D Renal tubular acidosis with organic aciduria during paraldehyde ingestion Am J Med 23 977 1957
20 Darmady E M and Stranack F Microdissection of the nephron in disease Brit M Bull 13 21 1957
21 Relman A S and Schwartz W B The kidney in potassium depletion Am J Med 24 764 1958
22 Relman A S What are acids and bases? Am J Med 17 435 1954
23 Kaufman H E and Rosen, S W Clinical acid base regulation—the Bronsted schema Surg Gynec & Obst 103 101 1956
24 Smith H W *The Kidney Structure and Function in Health and Disease* Oxford New York 1951
25 Gottschalk C W Mylle M and Lassiter W E Micropuncture evidence for proximal acidification of the urine Fed Proc 18 58 1959
26 Pitts R F and Lotspeich W D Bicarbonate and the renal regulation of acid base balance Am J Physiol 147 138 1946
27 Berliner R W Kennedy T J Jr and Orloff J Relationship between acidification of the urine and potassium metabolism Am J Med 11 274 1951
28 Schwartz W B Hall P W Hays R M and Relman A S On the mechanism of acidosis in chronic renal disease J Clin Invest 38 39 1959
29 Pitts R F and Alexander R S The nature of the mechanism for acidifying the urine Am J Physiol 144 239 1945
30 Davenport H W and Wilhelmi A E Renal carbonic anhydrase Proc Soc Exper Biol & Med 48 53 1941
31 Berliner R W and Orloff J Carbonic anhydrase inhibitors Pharmacol Rev 8 137 1956
32 Montgomery H and Pierce J A The site of acidification of the urine within the renal tubule in amphibia Am J Physiol 118 144 1937
33 Pitts R F Some reflections on mechanisms of action of diuretics Am J Med 24 745 1958
34 Van Slyke D D Phillips R A Hamilton P B Archibald R M Futcher P H and Hiller A Glutamine as source material of urinary ammonia J Biol Chem 150 48 1943
35 Orloff J and Berliner R W The mechanism of the excretion of ammonia in the dog J Clin Invest 35 223 1956

36 Lotspeich W D and Pitts R F The role of amino acids in the renal tubular secretion of ammonia J Biol Chem 168 611 1947
37 Madison L L and Seldin D W Ammonia excretion and renal enzymatic adaptation in human subjects as disclosed by administration of precursor amino acids J Clin Invest 37 1615 1958
38 Davies B M A and Yudkin J Studies in biochemical adaptation The origin of urinary ammonia as indicated by the effect of chronic acidosis and alkalosis on some renal enzymes in the rat Biochem J 52 407 1952
39 Rector F C Jr and Orloff J The effect of the administration of sodium bicarbonate and ammonium chloride on the excretion and production of ammonia The absence of alterations in the activity of renal ammonia-producing enzymes in the dog J Clin Invest 38 366 1959
40 Milne M D Scribner B H and Crawford M A Non ionic diffusion and the excretion of weak acids and bases Am J Med 24 709 1958
41 Latner A L and Burnard E D Idiopathic hyperchloremic renal acidosis of infants observations on the site and nature of the lesion Quart J Med (N S) 19 285 1950
42 Smith L H Jr and Schreiner G L Studies on hyperchloremic acidosis J Lab & Clin Med 43 347 1954
43 Schiess W A Ayer J L Lotspeich W D and Pitts R F The renal regulation of acid base balance in man II Factors affecting the excretion of titratable acid by the normal human subject J Clin Invest 27 57 1948
44 Kaye M The effect of a single oral dose of the carbonic anhydrase inhibitor acetazoleamide in renal disease J Clin Invest 34 277 1955
45 Harrison H E Mechanisms of action of vitamin D Pediatrics 14 285 1954
46 Harrison H E and Harrison H C Inhibition of urine citrate excretion and the production of renal calcinosis in the rat by acetazoleamide (Diamox) administration J Clin Invest 34 1662 1955
47 Harrison H E Chisolm J J Jr and Harrison H C Congenital renal tubular acidosis A M A J Dis Child 96 588 1958
48 Farrell G Regulation of aldosterone secretion Physiol Rev 38 709 1958
49 Dent C E and Harris H Hereditary forms of rickets and osteomalacia J Bone & Joint Surg 38 B 204 1956
50 Rendle-Short J Idiopathic renal acidosis in twins Alkalosis resulting from over dosage of a citrate mixture Arch Dis Childhood 28 55 1953
51 Cooke R E and Kleeman C R Distal tubular dysfunction with renal calcification Yale J Biol & Med 23 199 1950
52 Engel W J Nephrocalcinosis J A M A 145 288 1951
53 Gordon E E and Sheps S G Effect of acetazoleamide on citrate excretion and formation of renal calculi Report of a case and study of five normal subjects New England J Med 256 1215 1957

# Chapter 40

# Vasopressin resistant Diabetes Insipidus

*Jack Orloff and Maurice B Burg*

Vasopressin resistant diabetes insipidus is a rare congenital and familial disease characterized by failure of the kidneys to respond to antidiuretic hormone The essential features of the disorder are polyuria polydipsia and the excretion of persistently hypotonic urine The disease was first described by Forssman [*1*] and Waring et al [*2*] who recognizing the inefficacy of vasopressin in promoting the excretion of hypertonic urine distinguished it from diabetes insipidus due to insufficiency of neurohypophyseal hormone Later Williams and Henry [*3*] introduced the name *nephrogenic diabetes insipidus* in order to emphasize that insensitivity of the renal tubule cells to antidiuretic hormone is responsible for all the clinical findings in the disease

## PHYSIOLOGY OF WATER BALANCE

In normal man the osmotic pressure of plasma in essence a reflection of the ratio of water to solute in the body is virtually constant (285 to 295 mOsm per kg $H_2O$) despite wide variations in the intake of solute and water Regulation is achieved by varying both the volume flow of urine and its osmotic pressure in response to changes in plasma osmolality A fall in plasma osmolality indicative of relative water excess, is normally attended by the excretion of large volumes of urine less concentrated than plasma This loss of water without its equivalent content of solute (285 µOsm for every milliliter of water) restores plasma osmolality to normal Conversely hyperosmolality whether due to water loss or retention of solute in excess of water is corrected by the excretion of urine more concentrated than plasma

The precise adjustment of urine flow and osmolality is in large part due to the remarkable efficiency of the pituitary renal system The kidney is capable of varying urine flow under physiologic conditions in the adult

from less than 1 to more than 20 ml per min and urine concentration from a maximum of 1 400 to a minimum of 30 to 40 mOsm per kg $H_2O$ These changes are effected to a considerable degree by variations in the tubular reabsorption of water largely determined by the action of the neurohypophyseal hormone vasopressin

## ROLE OF THE NEUROHYPOPHYSIS IN WATER BALANCE

Vasopressin an octapeptide [4] is elaborated in the supraoptic and paraventricular nuclei of the hypothalamus [5] [1]

Vasopressin is thought to be stored in the posterior portion of the pituitary body from which it is secreted into the blood in response to nervous stimuli originating in cells ( osmoreceptors ) in the anterior hypothalamus and transmitted to the pituitary along the supraoptico hypophyseal tracts [6] On the basis of Verney s [6] classical demonstrations it has been concluded that shrinkage of the osmoreceptors due to an elevation in the effective osmotic pressure[2] of plasma effects the release of antidiuretic hormone (vasopressin) into the circulating blood The resultant increase in urine osmolality restores plasma osmolality to normal thereby diminishing the rate of secretion of hormone Conversely a reduction in effective osmotic pressure by virtue of swelling of the osmoreceptors leads to inhibition of antidiuretic hormone release and permits the excretion of large volumes of dilute urine It is important to recognize that graded release of hormone is essential in order to afford the continuous regulation of urine flow and concentration necessary to minimize deviations in plasma osmolality Although the renal regulation of water balance is inadequate in the absence of a properly functioning hypothalamicohypophyseal system or when the kidney is insensitive to the effect of antidiuretic hormone as in the disease under discussion the patient with either form of diabetes insipidus is capable of maintaining plasma osmolality within the normal range merely by adjusting fluid intake As long as the volume of water ingested exceeds that excreted in the urine by an amount equivalent to that dissipated in the form of

[1] The hormone isolated from ox pituitary by du Vigneaud and his associates is a cyclic octapept de with the follo ng stru ture [ ]



Cys Tyr Phe Glu(NH ) Asp(NH ) Cys Pro Arg Gly($NH_2$)

Hog vasopressin contains lysine in the penultimate position All other mammals studied includ ng man are thought to sec ete the arginine form depicted above

[2] Effective osmotic pressure is that exerted by olutes that do not penetrate cell membranes rapidly Sodium and attendant anions represent the major osmotically effective constituents of the extracellular space Urea on the other hand though it may contribute appreciably to the total osmotic pressure does not affect the distribution of water between cells and surrounding extracellular fluids since it penetrates cells freely and its con entrat on is appro imately equal in the two phases

insensible loss and perspiration excessive concentration of body fluids will not occur In this situation, thirst rather than variable reabsorption of water assumes a regulatory role

## RENAL REGULATION OF WATER BALANCE

Although the antidiuretic effect of vasopressin and the physiologic factors involved in its release from the hypophysis have been recognized for many years the intrarenal events which transpire in the elaboration of hypotonic or hypertonic urine are not yet completely understood

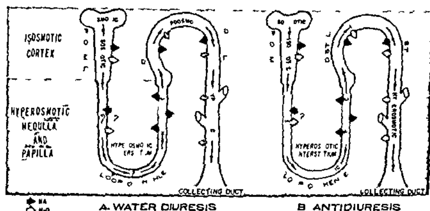



Fig 40-1 Schematic representation of the nephron during water diuresis (A) and antidiuresis (B) The diagram is described in detail under The Countercurrent Theory Although not indicated in the figure the longitudinal interstitial osmotic gradient increases progressively from corticomedullary junction to papilla The size of the arrows representing $H_2O$ and NaCl reabsorption indicates the relative extent of these processes The development of hyperosmolality in the descending limb of Henle s loop has been established only in antidiuresis That this also occurs in water diuresis is likely but unknown Furthermore although hyperosmolarity in the descending limb is probably the result of water abstraction the movement of urea and other solutes from the interstitial space into the lumen also may be an important factor The role of urea in the concentrating and diluting processes discussed in the text has been omitted from the figure

Sufficient data derived from a variety of sources are available to justify a tentative description of the process [7–13]

In order to account for the normal variations in urine flow and concentration the plasma ultrafiltrate formed at the glomerulus must be modified as it courses down the tubule Two fundamental processes are involved the selective abstraction of solute and the tubular reabsorption of water Water reabsorption in the tubule is divided into at least three and possibly four functionally and anatomically distinct phases On the basis of the most recent evidence it is believed that water diffuses*

* The term diffusion is used throughout to connote movement of water across the tubular membrane It should be recognized that this is inaccurate since bulk flow through pores along an osmotic gradient may account for most of the net movement [18]

passively out of the proximal segment the descending limb of Henle s loop the distal convoluted tubule and the collecting duct along osmotic gradients established by prior solute removal The proximal segment and descending limb of Henle s loop are considered to be freely permeable to water under all circumstances the distal convolution and collecting duct are freely permeable only in the presence of antidiuretic hormone Consequently variations in the permeability of the membrane to water in the latter segments impose restrictions on diffusion and permit the elaboration of urine of varying tonicity and volume Furthermore it has been established [14] that there is a progressive increase in the osmotic pressure of the renal interstitial fluid from cortex to papilla (Fig 40-1), the medulla and papilla being hyperosmotic to plasma under all circumstances whereas the cortex is isosmotic

Normally 20 per cent of the plasma perfusing the kidney is filtered at the glomerulus Of the ultrafiltrate 80 to 85 per cent is reabsorbed in the proximal segment essentially as an isosmotic solution [7 8 12 15] As noted above the luminal membrane in this area is freely permeable to water under all circumstances Sodium and attendant anion are reabsorbed by a process involving active sodium transport (i e transport against an electrochemical gradient) water then diffuses passively out of the tubule along the resultant osmotic gradient The reduced volume of isosmotic fluid is then delivered to the loop of Henle Although the sequence of events in this segment has not been established with certainty it is probable that Wirz s [11 14] original contention that the urine is progressively concentrated as it flows downward in the descending limb and is then diluted in the upward ascent in the ascending limb is correct In support of this are the observations (1) that urine at the tip of Henle s loop is hypertonic to plasma [12] [4] and (2) that urine in the first portion of the distal convolution and therefore presumably in the ascending limb of Henle s loop is hypotonic to plasma irrespective of the final urine concentration [12 15] In view of this it is reasonable to assume that the descending limb is freely permeable to water under all circumstances hypertonicity being effected by loss of water without solute into the surrounding hypertonic interstitium whereas the ascending limb is impermeable to water dilution being achieved by the active extrusion of sodium chloride without water It is essential to recognize that the processes described thus far are uninfluenced by vasopressin and occur as described whether or not the final urine is concentrated or dilute Were no other factors involved one could visualize the situation in vasopressin resistant diabetes insipidus (and in water diuresis in general) as the excretion of most of the residual hypotonic urine leaving

[4] This has been established in antidiuresis only No examinations of loop fluid during water diuresis have been reported In the context of this discussion it is assumed that hypertonicity in this segment is also developed in water diuresis

the loop of Henle That this is an oversimplification is attested to by arguments to be presented subsequently

The sequence of events in the distal convolution and collecting duct, unlike that in the proximal portions of the nephron, differs considerably in water diuresis and antidiuresis In the absence of vasopressin and when a dilute urine is to be excreted, the luminal membrane in the distal nephron is thought to be relatively impermeable to water Dilute urine which leaves Henle s loop is rendered more hypotonic by the continued abstraction of solute without water in the distal convolution and perhaps even in the collecting duct [16, 17] Consequently urine less concentrated than plasma is excreted into the bladder Although some loss of water along its osmotic gradient takes place in the distal nephron even in the absence of vasopressin, it is generally insufficient to effect the excretion of either an isotonic or hypertonic urine

In marked contrast, when vasopressin is present hypertonic urine is elaborated Antidiuretic hormone is thought to increase the permeability of the distal convolution [9–11] and collecting duct to water [11], so that for all intents and purposes these areas of the tubule function as do the proximal segment Although electrolyte is abstracted from hypotonic urine in the distal convolution just as in water diuresis water now diffuses freely out of the tubule until osmotic equilibrium is achieved The remaining urine once again isosmotic to plasma is delivered to the collecting duct where water in excess of solute is lost by movement into the hypertonic interstitium [15] The resultant concentrated urine is excreted

An effect of vasopressin on membrane permeability has been deduced from studies in other tissues Koefoed Johnsen and Ussing [18] observed a marked increase in net water movement along an osmotic gradient across isolated frog skin under the influence of neurohypophyseal extract They concluded that the hormone produces this effect by increasing the size of pores within the skin through which water may flow It is likely that vasopressin induces similar changes in membrane permeability in both the distal convolution and collecting duct Wirz [10] and Gottschalk and Mylle [12] noted that urine in the distal convolution which remains hypotonic throughout during water diuresis (when antidiuretic hormone secretion is in abeyance) becomes isosmotic in this segment in anti diuresis An analogous increase in the permeability of the collecting duct to water has also been suggested by Wirz [11] In the presence of anti diuretic hormone the osmotic pressure of collecting duct contents approximates that of the surrounding tissue and blood Consequently as noted above urine made dilute by solute abstraction in Henle s loop no longer flows through essentially water impermeable conduits in the distal nephron Instead water in excess of solute moves out of these areas ultimately resulting in the elaboration of hypertonic urine

The view that water movement in all segments of the nephron including that in the collecting duct is passive has only recently been proposed The terminal process which results in the elaboration of urine more concentrated than plasma had been assumed to be an active process since it apparently required uphill transport of water i e movement of water from a solution in which the activity of water is less than that of the surrounding interstitium The argument was based on the belief that the osmotic pressure of the renal interstitial space approximated that of all other tissues of the body and was 290 mOsm per kg $H_2O$ Wirz [14] resolved this problem and provided a rational basis for considering that water movement out of the tubule lumen is passive (along its activity gradient) even when urine is markedly hypertonic He observed by direct cryoscopic analysis that the solute concentration of kidney water increases progressively from cortex to papilla and that both the inner medulla and papilla are markedly hypertonic to peripheral plasma He concluded that the hypertonic environment surrounding the collecting duct provides the osmotic force necessary for passive movement of water out of this segment He was the first to suggest that the achievement of diffusion equilibrium when the membrane is made freely permeable to water by vasopressin results in the elaboration of hypertonic urine It is a testimony to Wirz s ingenuity to recall that hypertonicity of the medulla had been noted earlier [16] only to be ignored by most renal physiologists

*The Countercurrent Theory*

Wirz [15] considered the hypertonicity of the medulla and papilla (the longitudinal concentration gradient) to be due to deposition of sodium chloride transported out of the ascending limb of Henle s loop into the surrounding interstitium Although not explicitly stated as such in his original description it is likely as noted above that loss of water without solute in the descending limb raises the concentration of the urine in this segment above that of plasma This process concomitant concentration and dilution of urine in opposing segments of a loop like structure in which the flow of urine in one segment is counter to that of the other has a particular physiologic advantage Since the NaCl concentration of ascending limb urine is from the beginning approximately equal to that of the environment the transport of sodium salts out of this segment is nowhere against a steep concentration gradient This minimizes any energetic difficulties which might arise were sodium salts transported *from hypotonic urine to a markedly hypertonic environment* as has been suggested by Berliner et al [13]

Both the anatomic arrangement of the loop and the processes described have all the features of a hairpin countercurrent multiplier system The principle of the multiplier has been described in detail by Hargitay and Kuhn [19] and was originally applied in a somewhat modified fashion

to the kidney by these workers and Wirz [14] It is evident that deposition of NaCl in excess of water in the interstitial space of the medulla and papilla will raise the osmotic pressure of this area above that of the cortex Progressive multiplication of this longitudinal gradient to the extent observed experimentally is visualized in the following manner In a hypothetical first circulation through the loop the intraluminal fluid has the same concentration throughout equal to that of the cortex Active transport of NaCl from the water impermeable ascending limb lowers the concentration of ascending limb contents and raises that of the interstitial fluid and descending limb (see above) The slightly hypertonic descending limb fluid moves around through the hairpin bend in the loop into the ascending limb temporarily abolishing the concentration gradient between the opposing segments However, continuing transport of NaCl from the ascending limb now containing fluid more concentrated than in the previous circulation, raises the osmotic pressure of the interstitial and descending limb fluids once again Descending limb fluid more concentrated than previously moves into the ascending limb, and the process continues until in the steady state the longitudinal gradient is multiplied manyfold, whereas the small transverse gradient between loop contents and interstitium is at each point in the renal medulla equal and unchanged In other terms the hairpin countercurrent multiplier system, by permitting transport against a minimal gradient provides the basis for multiplying the small gradient between loop contents and interstitium manyfold in the longitudinal axis

Whether or not urinary dilution in the ascending section of the loop and concentration of the interstitial spaces occurs as described and is accomplished by a hairpin countercurrent multiplier device sequestration or trapping of NaCl in the interstitial space in order to maintain the concentration gradient cannot be explained simply on the basis of this hypothesis Since blood courses through this area one would expect the excess solute to be dissipated and the solute concentration never appreciably to exceed that of the inflowing blood This aspect discussed by Wirz [20] has been emphasized and developed in detail by Berliner et al [13] Noting that the postglomerular capillaries in this area do not course directly through the kidney but also bend back upon each other in the shape of loops they suggested that the capillaries may act as countercurrent exchangers This is illustrated in Fig 40-2 Free diffusion of solute from the outflowing to the inflowing limb of the capillary loop permits recirculation of solute in the medulla minimizing the loss of excess NaCl and other solutes (notably urea) from the area The concentration gradient is thereby maintained and not dissipated as it would be were blood flowing through in one direction only The capillary countercurrent exchanger not only maintains the interstitial NaCl concentration indirectly providing the osmotic force necessary for the terminal con

centrating process but also serves to trap any urea which diffuses into the interstitial space from the collecting duct [21] The concentration of urea in medullary water approaches that of collecting duct contents being approximately equal in antidiuresis In view of this Berliner et al [13] have pointed out that in so far as collecting duct urea is osmotically balanced by urea in the surrounding interstitial fluid it will increase the

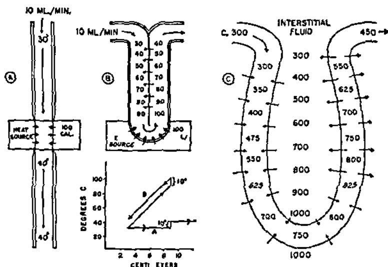



Fig 40-2 Principle of the countercurrent exchanger (*Rep oduc d fro i Berli er et al [13] with permission of the a itho and publi he s*)

A and B represent the effect of heating water flowing at the rate of 10 ml per min through a pipe The addition of 100 cal per min raises the temperature 10 in both A (straight flow) and B (countercurrent flow) However since the incoming water is heated by the outgoing water in B the maximum temperature attained in tl e counter current system is considerally higher than with straight flow The graph compares the temperature along the flow tubes in each system

C countercurrent flow as applied to the capillary loop showing that it is not neces sary for the limbs of the lo p to be in direct contact In the hypothetical illustration given both limbs are in contact with tl e ame interstitial fluid of progressively in crea ing concentration Sodium salts (arro s) at first enter the capillary blood later partly return to the interstitial flu d Note tl e analogy beween B in which heat is recirculated and C in which sodium salts are similarly retained in an area

osmotic pressure of urine beyond that attained by simple equilibration of collecting duct contents with interstitial NaCl In other terms the urine urea does not need to be balanced by an equivalent concentration of NaCl in the interstitial fluid as do the other solutes Consequently final urine osmolality will be greater or lesser depending on the urea load presented to the collecting duct even though no change in interstitial NaCl content occurs The clinical significance of this is considerable Inadequacy of urea load may limit the apparent concentrating ability of

the kidney, though no defect in either the permeability of the membrane to water or in the so-called sodium "pump" may be present [21, 22].

A schematic drawing of the nephron is illustrated in Fig 40-1. The cortex containing the glomerulus and the proximal and distal convolutions is represented as being isosmotic with peripheral plasma. The medulla and papilla, in which the loops of Henle, the collecting duct, and the deeper portions of the capillary loops are embedded, are represented as being hyperosmotic to plasma. Vasopressin affects only the distal convolution and the collecting duct. In diabetes insipidus or whenever vasopressin is either ineffective or not secreted, urine made dilute by transport of electrolyte out of both Henle's loop and the distal convolution loses only a small fraction of its water as it passes through the distal portions of the nephron and is excreted as such in the bladder. In the presence of vasopressin, free permeability of the distal convolution and collecting duct membrane to water permits osmotic flow of water out of the lumen. Consequently the osmolality of urine in each segment approximates that of the tubular environment, becoming isosmotic to plasma in the distal convolution and hyperosmotic in the collecting duct. It is important to recognize that the major effect of vasopressin in so far as water balance is concerned is in the distal convolution. The promotion of osmotic equilibrium in this area and the formation of isosmotic urine results in the restoration of as much as ±15 per cent of the filtered water to the body, whereas the reduction in volume in the collecting duct, though resulting in urine hypertonicity, restores considerably less (±5 per cent).

## FACTORS OTHER THAN VASOPRESSIN

### *Effects of Filtration Rate*

In order properly to evaluate the significance of changes in urine flow and osmolality in patients suspected of having vasopressin-resistant diabetes insipidus, it is essential to recognize the influence of at least two other factors, filtration rate and solute excretion, on the concentrating process. Berliner and Davidson [23] have demonstrated that a reduction in filtration rate may, even in the absence of vasopressin (or at least when its secretion is minimal and constant), promote the excretion of hypertonic urine. Presumably volume flow of hypotonic urine to the collecting duct is significantly diminished by this procedure. Consequently, although outward movement of water in the terminal segment is minimal under these circumstances, it may be sufficient to concentrate the small volume of urine delivered to the collecting duct. In view of this observation, minor increases in urine osmolality in diabetes insipidus following dehydration need not necessarily signify residual antidiuretic hormone activity. On the other hand, it is not at all certain that the elaboration of hypertonic

urine in patients with the pituitary disorder following protracted dehydration or manipulations designed to reduce filtration rate can uniformly be ascribed to the fall in filtration rate itself Residual antidiuretic hormone activity can never be completely excluded except in patients with the severe form of vasopressin resistant diabetes insipidus In one such patient although urine osmolality rose during dehydration in association with a fall in filtration rate it never exceeded that of plasma [24]

*Effects of Solute Excretion*

The influence of solute excretion on urine flow and concentration is clinically of greater significance than the effect of filtration rate Osmotic diuretics diminish water and solute reabsorption in the nephron and result in the excretion of large volumes of essentially isosmotic urine whether or not vasopressin is acting Both sodium transport and water reabsorption are interfered with in the proximal segment because of the osmotic restraint of nonabsorbed intraluminal solute Consequently a larger than normal volume of isosmotic urine enters the more distal portions of the nephron overwhelming the capacity of the segment to modify the concentration of the urine Urine osmolality rises in osmotic diuresis both in patients with nephrogenic diabetes insipidus [25] and in normal persons [26] during antidiuretic hormone suppression whereas in hydropenic subjects in whom vasopressin is acting the converse a progressive fall in osmolality occurs [27] In both instances urine osmolality approaches that of unmodified glomerular filtrate as the fraction of proximal urine delivered to the distal segment increases

*It is important to emphasize that no fundamental processes other than* proximal reabsorption need be disturbed during osmotic diuresis to account for the observations It is probable that in the presence of vasopressin more water is actually removed in the collecting duct during solute diuresis than at low rates of solute excretion [28] That this does not result in the achievement of maximal hypertonicity is due in part at least to the resultant dilution of the medullary interstitial space Even were osmotic equilibrium to obtain in this segment which is unlikely during the rapid flow of solute diuresis the maximal osmotic pressure which could be obtained would be considerably less than normal

The effects of osmotic diuresis are more easily visualized if one considers as do Wesson and Anslow [10] that hypotonic urine is made up of two hypothetical moieties (1) an isosmotic portion (referred to as the osmolar clearance ) equal in volume to the number of milliliters of fluid containing all the urine solute at the concentration of plasma and (2) an additional amount of distilled water (referred to as solute-free water) equal in volume to that which would have to be added *to the isosmotic moiety to reduce its* concentration to that observed Hypertonic urine on the other hand is

depicted as being made up of an isosmotic portion less that amount of solute-free water which would have to be abstracted from it to produce the observed hypertonicity (referred to as negative solute-free water) In this view, the removal of solute from isosmotic filtrate (the process of urinary dilution) results in the elaboration of solute free water, and conversely the abstraction of solute-free water from isosmotic filtrate results in the addition of negative-free water to the interstitium and the production of hypertonic urine Clearly, even were the amount of solute-free water formed in water diuresis or the amount of negative free water abstracted in antidiuresis unchanged in osmotic diuresis, urine concentration would approach isotonicity asymptotically with increasing urine flow since an increasingly greater fraction of the final urine would be composed of what may be considered nonabsorbed proximal isosmotic fluid

In the light of these concepts it is not possible to assess the clinical significance of changes in urine concentration without consideration of the rate of solute excretion In this regard the isosthenuria of renal insufficiency as has been pointed out by Baldwin et al [29] and Platt [30], may be a consequence of the high rate of solute excretion relative to the diminished filtration rate and does not necessarily denote either an inability to concentrate the urine or a vasopressin unresponsiveness The filtered load of urea per nephron is markedly increased in azotemia limiting the capacity of the kidney to elaborate either a maximally hyper tonic or hypotonic urine The extremes of urine osmolality are observed only when solute excretion per nephron is minimal

Even in the absence of antidiuretic hormone as noted above, water may diffuse out of the relatively impermeable distal segment and collecting duct although at a considerably lesser rate than when vasopressin is present [25 31] In the course of solute diuresis in vasopressin resistant diabetes insipidus Orloff and Walser [25] noted (Fig 40-3) not only a progressive rise in urine osmolality but also an increasingly greater rate of solute free water excretion They concluded that this phenomenon is in part due to a progressive decline in the rate of outward diffusion of water initially freed in Henle s loop and the distal convolution as a consequence of the osmotic restraint of intraluminal solute Thus the presence of solute in tubule urine decreases the activity of water and thereby its rate of outward diffusion in both the distal convoluted tubule and collecting duct Even in the presence of vasopressin massive solute diuresis may so limit outward diffusion of water as to result in the excretion of hypotonic urine Results of this nature have been observed during vasopressin administration in patients with pituitary diabetes insipidus [32] and in dogs undergoing combined water and solute diuresis [31] This is not a reflection of insensitivity to vasopressin but indicates that despite relatively free permeability to water in the distal nephron the reduction in both the transit time and the osmotic gradient prevents the attainment of diffusion equilibrium and permits the delivery of large volumes of

hypotonic rather than isotonic urine to the terminal concentrating site In view of these observations it is important to determine the normal relationship between urine osmolality and solute excretion before ascribing isosthenuria (see above) or even hyposthenuria[5] to true vasopressin insensitivity

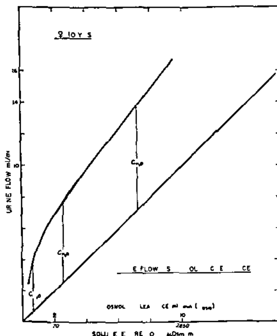


Fig 40-3 The relationship between urine flow and solute excretion in a 10-year-old girl with vasopressin resistant diabetes insipidus The diagonal line represents the excretion of isosmotic urine the curvilinear line that observed experimentally The difference between the hypothetical and observed lines represents solute-free water excretion ($C_{H_2O}$) which rose progressively throughout the study

## CLINICAL ASPECTS

Vasopressin resistant diabetes insipidus manifests itself soon after birth but since polyuria in infancy is rarely considered a symptom of disease even in the first born of the most apprehensive parent it is frequently undiagnosed in early life Furthermore the presenting signs which in fact may be alarming are often nonspecific being those associated with severe dehydration fever convulsions in infants vomiting constipation and hyperosmolality The characteristic syndrome—polydipsia polyuria and persistent hyposthenuria (specific gravity generally

[5] The excretion of urine less concentrated than plasma

less than 1 005[a])—diagnostic in offspring of affected families is noted only when hydration is adequate Under these circumstances, other causes of dehydration can generally be excluded

RESPONSE TO VASOPRESSIN

In contrast to what occurs in diabetes insipidus of pituitary origin, neither the copious urine flow nor the low urine concentration is affected by the administration of large doses of vasopressin (Table 40 1) Nor is it

TABLE 40-1 LACK OF EFFECT OF PURIFIED VASOPRESSIN IN A 1[illegible] YEAR-OLD MALE WITH VASOPRESSIN RESISTANT DIABETES INSIPIDUS

| Time min | V ml/min | $U_{osm}$ | $P_{osm}$ | GFR ml/min |
|---|---|---|---|---|
| 0 | Infuse 10 mg/min inulin in 6 1 ml/min 5% dextrose | | | |
| 50–70 | 10 2 | 44 | [illegible] | 83 |
| 70–90 | 10 8 | 48 | 293 | 87 |
| 130 | Infuse [illegible]0 mU/min vasopressin | | | |
| 130–1[illegible]0 | 11 1 | 44 | 290 | 87 |
| 1[illegible]0–170 | 10 7 | 48 | 287 | 89 |

Note V = urine flow $U_{osm}$ and $P_{osm}$ = urine and plasma osmolality mOsm/kg $H_2O$ GFR = glomerular filtration rate

possible to effect a diminution in flow or the elaboration of a hypertonic urine by stimuli known to enhance secretion of vasopressin from the pituitary body This is illustrated in Table 40-2 Although severe dehydration may ultimately result in a rise in urine osmolality and an associated fall in urine flow *urine hypertonicity has not been observed in patients totally unresponsive to vasopressin under these circumstances [24 33]*

Mild forms of the disease, characterized by partial responsiveness to vasopressin may exist in females of affected families Carter and Simp

[a] Specific gravity measurements have been used for many years to estimate urine concentration Since the former is a measure of urine density rather than of solute concentration and is dependent on both the weight and volume displacement of urine solids it cannot be used as a precise estimate of osmotic pressure This can be determined only by measuring the concentration of osmotically active constituents in urine by determining either *the freezing point or vapor pressure of the urine* Despite this there is a reasonably good correlation between urine specific gravity and osmotic pressure Significant discrepancies are observed only when abnormal solutes (glucose protein) constitute a large fraction of the total urine solute Under these circumstances specific gravity gives a falsely high estimate of urine concentration The converse a low estimate has been reported by Miles et al [34] when urea is present in high concentration in urine The limits of urine osmolality as determined by cryoscopic analysis have been referred to earlier Patients with nephrogenic diabetes insipidus generally cannot elaborate urine more concentrated than 50 to 100 mOsm although during solute diuresis urine osmolality may approach isotonicity (280 mOsm) as a limit Urine specific gravity is less than 1 005 approaching 1 010 during osmotic diuresis

kiss [35] observed that female siblings and relatives of patients with the full blown disorder were able to concentrate their urine to approximately 1 018 to 1 019 following 12 hr of dehydration Although this is significantly less than normal it does represent a considerable degree of responsiveness *Forssman [1] has reported similar findings Furthermore* Childs and Sidbury [36] have stated that two mothers of patients with the disease responded minimally to exogenous vasopressin Neither was able to elaborate a hypertonic urine Apparently although full expression of the renal defect may occur in both females and males incomplete expressivity has thus far been reported only in females

TABLE 40-2 ABSENCE OF EFFECT OF DEHYDRATION IN 12 YEAR-OLD MALE WITH VASOPRESSIN RESISTANT DIABETES INSIPIDUS

| *Time min* | *V ml/min* | *$U_{osm}$* | *$P_{osm}$* | *GFR ml/min* |
|---|---|---|---|---|
| 0 | Weight = 32 9 kg | | | |
| 60 | Infuse 20 ml/min inulin in 1 06 ml/min 5% dextrose in $H_2O$ | | | |
| 100–164 | 6 2 | 84 | 28[illegible] | 69 |
| 164–186 | 5 7 | 81 | 285 | 71 |
| 186–227 | 7 3 | 76 | 2[illegible]3 | 85 |
| 227–267 | 7 7 | [illegible] | 293 | [illegible]9 |
| 267–307 | 7 6 | 7[illegible] | 2[illegible] | 80 |
| 308 | Weight = 30 [illegible] kg | | | |

Note V = urine flow $U_{osm}$ and $P_{osm}$ = urine and plasma osmolality mOsm/kg $H_2O$ GFR = glomerular filtration rate

In all forms of the disease dehydration and its consequences are the most frequent complications as well as the most common causes of death The high infant mortality in affected families is undoubtedly ascribable *to this* The reported association of hydronephrosis and bladder hypertrophy in some affected individuals is probably secondary to polyuria and voluntary retention

## MENTAL AND PHYSICAL RETARDATION

A number of authors have commented on the relatively high incidence of mental and physical retardation in children with this disorder [3 37–42] It *is not at all clear that these are primary accompaniments of the* renal defect More likely structural brain damage if present is secondary to repeated episodes of severe dehydration [38 39] and to the altered growth pattern occasioned by inadequate food intake because of the exhausting influence of uncontrolled polydipsia Hillman et al [42] observed that an infant suffering from the disease became so exhausted from polydipsia when subsisting on a regular diet that he was unable to eat and play normally Mental performance and nutritional status were

improved by altering the protein and salt content of the diet so as to decrease obligatory solute and water loss. The reduction in polyuria diminished the time required for drinking and enabled the child to play, eat, and sleep adequately.

### RENAL FUNCTION

The only physiologic abnormality present in the disease is defective water reabsorption. In all other respects renal function may be considered to be normal. Renal blood flow, glomerular filtration rate, glucose, phosphate and amino acid transport, as well as other discrete tubular functions may be unaffected [2, 3, 25, 37, 41]. Changes in filtration rate and plasma flow which have been observed by some authors may have been due either to dehydration or associated but unrelated structural disease. Abnormalities of acid base balance and electrolyte metabolism are excluded by the normal plasma electrolyte patterns observed when hydration is adequate.

## PATHOGENESIS

The pathogenesis of the disease is unknown. Antidiuretic substance has been found in the blood [41, 43] and urine [40, 44, 45] of patients indicating that endogenous hormone is produced. Furthermore the administration of large doses of vasopressin, which is without effect on urine flow and concentration, results in abdominal pain and blanching, known side effects of the hormone [41, 44, 46, 47]. This indicates that rapid inactivation of the hormone within the body is not responsible for the disease. No information is available relative to the normal in vivo metabolism of vasopressin in man. It has not been ascertained whether the hormone may be inactivated in an abnormal fashion by the pertinent tubule cells. In view of recent evidence that vasopressin is bound to renal tissue during antidiuresis [48], it is possible that normal "binding" does not occur in vasopressin resistant diabetes insipidus. Other preparations of vasopressin, the synthetic arginine and lysine forms, are also ineffective [47].

No structural basis for the disorder has been found. Gross and microscopic examination of renal tissue by the usual techniques has not been revealing [2, 37, 40, 41, 49]. Although MacDonald [41] reported some shortening of the proximal segment in a microdissected specimen from one child, Darmady [49], who has examined one infant and two adults with the disease, has not been able to find any abnormality by microdissection or by histologic techniques.

On the basis of all the studies, it is assumed that end-organ unresponsiveness (presumably in the distal convolution and collecting duct) is responsible for the inefficacy of the hormone. The resultant fixed im

permeability of the distal nephron to water probably does not differ from that normally occurring in man during water diuresis since the relationship between solute excretion and urine osmolality is the same in both groups [47]

## DIAGNOSIS

The diagnosis is established without difficulty in the adequately hydrated subject in whom no response to vasopressin is observed Other causes of isosthenuria or hyposthenuria may be mistaken for the incompletely expressed form of the disease However the characteristic findings of the underlying disorder should preclude errors in diagnosis Diminished ability to concentrate urine maximally has been observed in hypokalemic nephropathy [50] hypercalcemia [51] sickle cell disease [52] so-called water losing nephritis [53] and postobstructive uropathy [54] In all but the last of these although the urine is hypertonic to plasma following vasopressin it is not maximally concentrated Isosthenuria in renal insufficiency is due in large part to solute diuresis (see above) and is generally not attended by true unresponsiveness to vasopressin Although it is possible that some patients may have structural disease with resultant insensitivity to vasopressin this has not been established Whether the reports of decreased concentrating ability in tubular acidosis [55] and in congenital tubular defects associated with aminoaciduria [56] are indicative of true unresponsiveness to vasopressin cannot be established on the basis of the available evidence

Psychogenic polydipsia may be associated with diminished responsiveness to vasopressin [57] DeWardener [58] observed a reversible limitation in concentrating ability in these and in normal subjects following prolonged chronic and excessive water ingestion DeWardener [57] has also stated that patients with the pituitary disorder may demonstrate this on occasion The cause of the temporary alteration in tubule permeability is unknown Prolonged administration of vasopressin and water in both man and dog may also be associated with an apparent diminution in responsiveness to the hormone [59–61]

### *Hereditary Pituitary Diabetes Insipidus*

Although the distinction between pituitary diabetes insipidus and the renal form generally is not difficult mild cases of vasopressin resistant diabetes insipidus have been confused with hereditary pituitary diabetes insipidus [1] The latter disorder is familial and also appears in early infancy However it is a less severe disease than the fully expressed form of vasopressin resistant diabetes insipidus Consequently dehydration and retarded development are uncommon Nonspecific atrophy of the posterior lobe of the pituitary and hypoplasia of the paraventricular and

improved by altering the protein and salt content of the diet so as to decrease obligatory solute and water loss. The reduction in polyuria diminished the time required for drinking and enabled the child to play, eat, and sleep adequately.

## RENAL FUNCTION

The only physiologic abnormality present in the disease is defective water reabsorption. In all other respects renal function may be considered to be normal. Renal blood flow, glomerular filtration rate, glucose, phosphate and amino acid transport, as well as other discrete tubular functions may be unaffected [2, 3, 25, 37, 41]. Changes in filtration rate and plasma flow which have been observed by some authors may have been due either to dehydration or associated but unrelated structural disease. Abnormalities of acid-base balance and electrolyte metabolism are excluded by the normal plasma electrolyte patterns observed when hydration is adequate.

permeability of the distal nephron to water probably does not differ from that normally occurring in man during water diuresis since the relationship between solute excretion and urine osmolality is the same in both groups [47]

## DIAGNOSIS

The diagnosis is established without difficulty in the adequately hydrated subject in whom no response to vasopressin is observed Other causes of isosthenuria or hyposthenuria may be mistaken for the incompletely expressed form of the disease However the characteristic findings of the underlying disorder should preclude errors in diagnosis Diminished ability to concentrate urine maximally has been observed in hypokalemic nephropathy [50] hypercalcemia [51] sickle cell disease [52] so called water losing nephritis [53] and postobstructive uropathy [54] In all but the last of these although the urine is hypertonic to plasma following vasopressin it is not maximally concentrated Isosthenuria in renal insufficiency is due in large part to solute diuresis (see above) and is generally not attended by true unresponsiveness to vasopressin Although it is possible that some patients may have structural disease with resultant insensitivity to vasopressin this has not been established Whether the reports of decreased concentrating ability in tubular acidosis [55] and in congenital tubular defects associated with aminoaciduria [56] are indicative of true unresponsiveness to vasopressin cannot be established on the basis of the available evidence

Psychogenic polydipsia may be associated with diminished responsiveness to vasopressin [57] DeWardener [58] observed a reversible limitation in concentrating ability in these and in normal subjects following prolonged chronic and excessive water ingestion DeWardener [57] has also stated that patients with the pituitary disorder may demonstrate this on occasion The cause of the temporary alteration in tubule permeability is unknown Prolonged administration of vasopressin and water in both man and dog may also be associated with an apparent diminution in responsiveness to the hormone [59–61]

### *Hereditary Pituitary Diabetes Insipidus*

Although the distinction between pituitary diabetes insipidus and the renal form generally is not difficult mild cases of vasopressin resistant diabetes insipidus have been confused with hereditary pituitary diabetes insipidus [1] The latter disorder is familial and also appears in early infancy However it is a less severe disease than the fully expressed form of vasopressin resistant diabetes insipidus Consequently dehydration and retarded development are uncommon Nonspecific atrophy of the posterior lobe of the pituitary and hypoplasia of the paraventricular and

supraoptic nuclei have been observed at autopsy According to Forssman [1] two forms of this disease exist, one transmitted as a sex linked recessive the other as an autosomal dominant

## GENETICS

The renal form of diabetes insipidus is an inherited disease which appears with greater frequency in male members of affected families than in females It was originally considered by Forssman [1] and Williams and Henry [3] to be transmitted as a sex linked recessive characteristic All of the 17 patients examined by these authors were males whose parents and female siblings were thought to be unaffected Furthermore the female siblings repeatedly passed the trait on to their male offspring No example of male to-male transmission was observed It has since been established that the disease is not completely recessive in female heterozygotes [35] Although all the males thus far reported exhibit complete unresponsiveness to vasopressin, mild forms of the disease have been observed in female siblings and relatives of affected males [1, 35] In addition in families in which there is no evidence that the fathers were affected several females have been reported in whom the disease is fully as severe as in males [25 44, 62, 65] On the basis of these observations it appears that nephrogenic diabetes insipidus is a dominant sex linked disorder with variable expressivity in heterozygous females If this is so mothers and half of the sisters of affected males should either be carriers of the defect or exhibit some form of the disease More careful studies in the future may establish this In the only affected families studied with this in mind 12 of 17 females examined were unable to *concentrate their urine normally following dehydration* [35] The discrepant preponderance of male patients in other sibships may indicate either a low degree of penetrance in females a failure to recognize cases with low expressivity or lowered viability and fertility of those affected males whose daughters would otherwise provide the excess female morbidity The severity of the symptoms in all the males reported in the literature may be indicative of a high degree of expressivity in this sex However no systematic study has excluded the possibility that male hemizygotes may show variable expressivity between and within kindreds as do heterozygous females

Childs and Sidbury [36] although conceding that the disease is most reasonably viewed as due to a gene occupying the X chromosome and showing perhaps intermediate dominance have suggested an alternative hypothesis i e autosomal dominance with some degree of sex limitation The latter view of the mode of inheritance has been emphasized by Cannon [62] He described a large pedigree in which 4 of 55 affected males were reported *to have affected sons* Cannon explained this pedigree by assuming that in some kindreds at least the disease is an autosomal

dominant with low penetrance in females Allen [64] has pointed out that Cannon s pedigree conforms closely to sex linked inheritance with the exception of the 4 cases of male to male transmission If inheritance were autosomal half the male offspring of affected males should have the disease and only half the female offspring of such males should be carriers According to Cannon s pedigree chart only 6 of the 44 male children were affected and of 19 females who had children 16 were affected or had affected children Either of these discrepancies alone is sufficient to preclude an autosomal theory of inheritance in this family

## TREATMENT

There is no specific therapy for the disease Adequate hydration easily achieved in the adult by allowing free access to water is essential to prevent the deteriorating effect of repeated episodes of dehydration Obligatory water loss may be minimized by reducing solute intake [39–42] This however is rarely necessary except in infants It has been reported recently that chlorothiazide a potent diuretic may induce satisfactory remissions in this disorder [65]

The therapy of the acute episode of dehydration may differ from that in other situations If sufficient water can be administered orally hyperosmolality of body fluids is easily overcome On the other hand if parenteral therapy is necessary 5 per cent dextrose in water although the fluid of choice in other instances of dehydration may aggravate the hypertonicity Orloff and Walser [47] observed that the rapid infusion of isosmotic dextrose in water in nephrogenic diabetes insipidus in so far as it produces solute diuresis (glycosuria) will so increase solute free water excretion as to promote the development of hyperosmolality and cellular dehydration The dextrose is not metabolized with sufficient speed to release solute-free water in excess of that excreted in the urine It is apparently virtually impossible to maintain positive water balance if 5 per cent dextrose is administered to patients with this disorder Successful correction of hyperosmolality may be accomplished by administering 2½ to 3 per cent dextrose in water [7] since under these circumstances it is possible to limit the extent of the urinary solute and water loss It is advisable to administer water without solute orally as soon as is clinically feasible

## SUMMARY

1 Vasopressin resistant diabetes insipidus is a renal disorder characterized by polydipsia polyuria and the excretion of persistently hypotonic urine It is distinguished from diabetes insipidus due to in

Intravascular hemolysis is not observed if 2½ to 3 per cent dextrose in water is administered parenterally Less concentrated solutions may be hazardous

sufficiency of antidiuretic hormone by a lack of response to exogenous vasopressin

2 All males thus far reported are completely unresponsive to antidiuretic hormone Mild forms of the disease, in which a limited response to vasopressin occurs, are observed in some female members of affected families

3 The only known defect present in the disease is insensitivity of the renal tubule cells to vasopressin No anatomic or biochemical basis for the end organ unresponsiveness has been determined The hormone normally accelerates the passive reabsorption of water in the distal nephron ultimately resulting in a reduction in urine flow and a rise in urine osmolality In its absence, or when the pertinent tubule cells are insensitive to its effect water diuresis is continuous

4 The only established treatment is maintenance of water balance by ingestion of adequate amounts of water In infants it is sometimes necessary to reduce the water requirement by restricting solute intake Life expectancy is normal if episodes of dehydration are prevented by permitting free access to water under all circumstances Chlorothiazide has induced remissions in some cases

5 The genetic pattern of the disorder is consistent with transmission as a sex linked dominant characteristic with variable expressivity in females The suggestion that an autosomal form of inheritance may be present in some families is not fully supported by the available data

## BIBLIOGRAPHY


1 Forssman H On hereditary diabetes insipidus With special reference to a sex linked form Acta med scandinav **121** Suppl [illegible] 1 1956
2 Waring A J Kajdi L and Tappan V A congenital defect of water metabolism Am J Dis Child **69** 323 1945
3 Williams R H and Henry C Nephrogenic diabetes insidipus transmitted by females and appearing during infancy in males Ann Int Med **27** 84 1947
4 du Vigneaud V Lawler H C and Popenoe L A Enzymatic cleavage of glycinamide from vasopressin and a proposed structure for this pressor antidiuretic hormone of the posterior pituitary J Am Chem Soc **75** 4880 1953
5 Scharrer E and Scharrer B Hormones produced by neurosecretory cells Recent Progr Hormone Res **10** 183 1954
6 Verney E B Renal excretion of water and salt Lancet **2** 1237 and [illegible] 1957
7 Richards A N The Croonian Lecture Processes of urine formation Proc Roy Soc London Series B **126** 398 1938
8 Walker A M Bott P A Oliver J and MacDowell M C The collection and analysis of fluid from single nephrons of the mammalian kidney Am J Physiol **134** 580 1941
9 Smith H W *The Kidney Structure and Function in Health and Disease* Oxford New York 1951
10 Wesson L G Jr and Anslow W P Effect of osmotic and mercurial diuresis on simultaneous water diuresis Am J Physiol **170** 255 1952
11 Wirz H The localization of antidiuretic action in the mammalian kidney in *The Neurohypophysis* edited by H Heller p 157 Academic Press Inc New York 1957

12 Gottschalk C W and Mylle M Micropuncture study of the mammalian urinary concentrating mechanism evidence for the countercurrent hypothesis Am J Physiol 196 927 1959
13 Berliner R W Levinsky N G Davidson D G and Eden M Dilution and concentration of the urine and the action of antidiuretic hormone Am J Med 24 730 1958
14 Wirz H Hargitay B and Kuhn W Lokalisation des Konzentrierungsprozesses in der Niere durch direkte Kryoskopie Helvet physiol et pharmacol acta 9 196 1951
15 Wirz H Der osmotische Druck in den corticalen Tubuli der Rattenniere Helvet physiol et pharmacol acta 14 353 1956
16 Ljungberg E On the reabsorption of chlorides in the kidney of the rabbit Acta med scandinav Suppl 127 186 1 1947
17 Hilger H H Klumper J D and Ullrich K J Reabsorption of water and ion transport by the cells of the collecting ducts of the mammalian kidney (microanalytical studies) Pflugers Arch ges Physiol 267 218 1958
18. Koefoed Johnsen V and Ussing H H The contributions of diffusion and flow to the passage of H O through living membranes Effect of neurohypophyseal hormone on isolated Anuran skin Acta physiol scandinav 28 60 1953
19 Hargitay B and Kuhn W Das Multiplikationsprinzip als Grundlage der Harnkonzentrierung in der Niere Ztschr Elektrochem 55 539 1951
20 Wirz H Der osmotische Druck des Blutes in der nieren Papille Helvet physiol et pharmacol acta 11 20 1953
21 Levinsky N G and Berliner R W The role of urea in the urine concentrating mechanism J Clin Invest 38 741 1959
22 Epstein F H Kleeman C R Pursel S and Hendrikx A The effect of feeding protein and urea on the renal concentrating process J Clin Invest 36 635 1957
23 Berliner R W and Davidson D G Production of hypertonic urine in the absence of pituitary antidiuretic hormone J Clin Invest 36 1416 1957
24 Keitel H G Walser M and Orloff J Unpublished data
25 Orloff J and Walser M Water and solute excretion in pitressin resistant diabetes insipidus Clin Res Proc 4 136 1956
26 Kleeman C R Epstein F H and White C The effect of variations in solute excretion and glomerular filtration on water diuresis J Clin Invest 35 749 1956
27 McCance R A The excretion of urea salts and water during periods of hydropenia in man J Physiol 104 196 1945
28 Page L B and Reem G H Urinary concentrating mechanism in the dog Am J Physiol 171 572 1952
29 Bald in D S Berman H J Heinemann H O and Smith H W The elaboration of osmotically concentrated urine in renal disease J Clin Invest 34 800 1955
30 Platt R Renal failure Lancet 1 1239 1951
31 Orloff J Wagner H N and Davidson D G The effect of variations in solute excretion and vasopressin dosage on the excretion of water in the dog J Clin Invest 37 4 8 1958
32 de Wardener H E and del Greco F The influence of solute excretion rate on the production of a hypotonic urine in man Clin Sc 14 715 1955
33 Childs B Personal communication
34 Miles B E Paton A and de Wardener H E Maximum urine concentration Brit M J 2 901 1954
35 Carter C and Simpkiss M The carrier state in nephrogenic diabetes insipidus Lancet 2 1069 1956
36 Childs B and Sidbury J B A survey of genetics as it applies to problems in medicine Pediatrics 20 177 1957
37 Kirman B H Black J A Wilkinson R H and Evans P R Familial pitressin resistant diabetes insipidus with mental defect Arch Dis Childhood 31 59 1956
38 Lilborg A and Forssman H Nephrogenic diabetes insipidus in children Acta paediat 44 09 1955

sufficiency of antidiuretic hormone by a lack of response to exogenous vasopressin

2 All males thus far reported are completely unresponsive to antidiuretic hormone Mild forms of the disease in which a limited response to vasopressin occurs, are observed in some female members of affected families

3 The only known defect present in the disease is insensitivity of the renal tubule cells to vasopressin No anatomic or biochemical basis for the end organ unresponsiveness has been determined The hormone normally accelerates the passive reabsorption of water in the distal nephron, ultimately resulting in a reduction in urine flow and a rise in urine osmolality In its absence or when the pertinent tubule cells are insensitive to its effect water diuresis is continuous

4 The only established treatment is maintenance of water balance by ingestion of adequate amounts of water In infants it is sometimes necessary to reduce the water requirement by restricting solute intake Life expectancy is normal if episodes of dehydration are prevented by permitting free access to water under all circumstances Chlorothiazide has induced remissions in some cases

5 The genetic pattern of the disorder is consistent with transmission as a sex linked dominant characteristic with variable expressivity in females The suggestion that an autosomal form of inheritance may be present in some families is not fully supported by the available data

## BIBLIOGRAPHY


1 Forssman H On hereditary diabetes insipidus With special reference to a sex linked form Acta med scandinav 121 Suppl [illegible] 1 [illegible]

2 Waring A J Kajdi L and Tappan V A congenital defect of water metabolism Am J Dis Child 69 323 1945

3 Williams R H and Henry C Nephrogenic diabetes insidipus transmitted by females and appearing during infancy in males Ann Int Med 27 84 1947

4 du Vigneaud V Lawler H C and Popenoe E A Enzymatic cleavage of glycinamide from vasopressin and a proposed structure for this pressor antidiuretic hormone of the posterior pituitary J Am Chem Soc 75 4880 1953

5 Scharrer E and Scharrer B Hormones produced by neurosecretory cells Recent Progr Hormone Res 10 183 1954

6 Verney E B Renal excretion of water and salt Lancet 2 1237 and 1295 1957

7 Richards A N The Croonian Lecture Processes of urine formation Proc Roy Soc London Series B 126 398 1938

8 Walker A M Bott P A Oliver J and MacDowell M C The collection and analysis of fluid from single nephrons of the mammalian kidney Am J Physiol 134 580 1941

9 Smith H W *The Kidney Structure and Function in Health and Disease* Oxford New York 1951

10 Wesson L G Jr and Anslow W P Effect of osmotic and mercurial diuresis on simultaneous water diuresis Am J Physiol 170 255 [illegible]

11 Wirz H The localization of antidiuretic action in the mammalian kidney in *The Neurohypophysis* edited by H Heller p 157 Academic Press Inc New York 1957

12 Gottschalk C W and Mylle M Micropuncture study of the mammalian urinary concentrating mechanism evidence for the countercurrent hypothesis Am J Physiol 196 9?7 19?J
13 Berliner R W Levinsky N G Davidson D G and Eden M Dilution and concentration of the urine and the action of antidiuretic hormone Am J Med 24 0 19 8
14 Wirz H Hargitay B and Kuhn W Lokalisation des Konzentrierungsprozesses in der Niere durch direkte Kryoskopie Helvet physiol et pharmacol acta 9 196 1951
15 Wirz H Der osmotische Druck in den corticalen Tubuli der Rattenniere Helvet physiol et pharmacol acta 14 353 1956
16 Ljungberg E On the reabsorption of chlorides in the kidney of the rabbit Acta med scandinav Suppl 127 186 1 1947
17 Hilger H H Klümper J D and Ullrich K J Reabsorption of water and ion transport by the cells of the collecting ducts of the mammalian kidney (micro analytical studies) Pflugers Arch ges Physiol 267 218 1958
18 Koefoed-Johnsen V and Ussing H H The contributions of diffusion and flow to the passage of $H_2O$ through living membranes Effect of neurohypophyseal hormone on isolated Anuran skin Acta physiol scandinav 28 60 1953
19 Hargitay B and Kuhn W Das Multiplikationsprinzik als Grundlage der Harnkonzentrierung in der Niere Ztschr Elektrochem 55 539 1951
?0 Wirz H Der osmotische Druck de Blutes in der nieren Papille Helvet physiol et pharmacol acta 11 20 1953
21 Levinsky N G and Berliner R W The role of urea in the urine concentrating mechanism J Clin Invest 38 741 1959
22 Epstein F H Kleeman C R Pursel S and Hendrikx A The effect of feeding protein and urea on the renal concentrating process J Clin Invest 36 635 1957
?3 Berliner R W and Davidson D G Production of hypertonic urine in the absence of pituitary antidiuretic hormone J Clin Invest 36 1416 1957
24 Keitel H G Walser M and Orloff J Unpublished data
25 Orloff J and Walser M Water and solute excretion in pitressin resistant diabetes insipidus Clin Res Proc 4, 136 1956
6 Kleeman C R Epstein F H and White C The effect of variations in solute excretion and glomerular filtration on water diuresis J Clin Invest 35 749 1956
27 McCance R A The excretion of urea salts and water during periods of hydropenia in man J Physiol 104 196 1945
8 Page L B and Reem G H Urinary concentrating mechanism in the dog Am J Physiol 171 5/2 1952
29 Baldwin D S Berman H J Heinemann H O and Smith H W The elaboration of osmotically concentrated urine in renal disease J Clin Invest 34 800 1955
30 Platt R Renal failure Lancet 1 1239 1951
31 Orloff J Wagner H N and Davidson D G The effect of variations in solute excretion and vasopressin dosage on the excretion of water in the dog J Clin Invest 37 458 1958
3 de Wardener H E and del Greco F The influence of solute excretion rate on the production of a hypotonic urine in man Clin Sc 14 715 1955
33 Childs B Personal communication
34 Miles B E Paton A and de Wardener H E Maximum urine concentration Brit M J 2 901 1954
35 Carter C and Simpkiss M The carrier tate in nephrogenic diabetes insipidus Lancet 2 1069 1956
36 Childs B and Sidbury J B A survey of genetics as it applies to problems in medicine Pediatrics 20 177 1957
37 Kirman B H Black J A Wilkinson R H and Evans P R Familial pitressin resistant diabetes insipidus with mental defect Arch Dis Childhood 31 59 19 6
38 Ellborg A and Forssman H Nephrogenic diabetes insipidus in children Acta paediat 44 ?09 1955

39 Gautier L and Simpkiss M The management of nephrogenic diabetes insipidus in early life Acta paediat 46 354 1957
40 Wattiez I R Loeb H Bellens R and van Geffel R Diabète insipide pitressino-résistant Helvet paediat acta 12 643 1957
41 MacDonald W B Congenital pitressin resistant diabetes insipidus of renal origin Pediatrics 15 298 1955
42 Hillman D A Oleay N Porter P Cushman A and Talbot N B Renal (vasopressin resistant) diabetes insipidus Definition of the effects of a homeostatic limitation in capacity to conserve water on the physical intellectual and emotional development of a child Pediatrics 21 430 1958
43 Linneweh F Buchborn E and Dellbruck B Familiarer renaler Diabetes Insipidus Klin Wchnschr 35 22 1957
44 Dancis J Birmingham J R and Leslie S H Congenital diabetes insipidus resistant to treatment with pitressin Am J Dis Child 75 316 1948
45 Luder J and Burnett D A congenital renal tubular defect Arch Dis Childhood 29 44 1954
46 Kao M Y C and Steiner M M Diabetes insipidus in infancy resistant to pitressin Pediatrics 12 400 1953
47 Orloff J and Walser M Unpublished observations
48 Schwartz I L Fong C T O Popenoe E A Silver L and Schoessler M A Evidence for a covalent attachment of the antidiuretic hormone to its receptor site in the kidney J Clin Invest 38 1041 1959
49 Darmady E M Personal communication
50 Relman A S and Schwartz W B The kidney in potassium depletion Am J Med 24 764 1958
51 Cohen S I Fitzgerald M G Fourman P Griffeths W J and de Wardener H E Polyuria in hyperparathyroidism Quart J Med 26 423 1957
52 Keitel H G Thompson D and Itano H A Hyposthenuria in sickle cell anemia a reversible renal defect J Clin Invest 35 998 1956
53 Roussak N J and Oleesky S Water losing nephritis A syndrome simulating diabetes insipidus Quart J Med 23 147 1954
54 Earley L E Extreme polyuria in obstructive uropathy Report of a case of water losing nephritis in an infant with a discussion of polyuria New England J Med 255 600 1956
55 Cooke R E and Kleeman C R Distal tubular dysfunction with renal calcification Yale J Biol & Med 23 199 1950
56 Bickel H Baar H S Astley R Douglas A A Finch E Harris H Harvey C C Hickmans E M Philpott M G Smallwood W C Smellie J M and Teall C G Cystine storage disease with aminoaciduria and dwarfism (Lignac Fanconi disease) Acta paediat 42 Suppl 90 1 1952
57 de Wardener H E *The Kidney* Little Brown Boston 1958
58 de Wardener H E and Herxheimer A The effect of a high water intake on the kidney s ability to concentrate the urine in man J Physiol 139 42 1957
59 Jaenike J R and Waterhouse C The effects of sustained vasopressin administration in man Clin Res Proc 7 272 1959
60 Davis J O Howell D S and Hyatt R E Effect of chronic pitressin administration on electrolyte excretion in normal dogs and in dogs with experimental ascites Endocrinology 55 409 1954
61 Levinsky N G Davidson D G and Berliner R W Changes in urine concentration during prolonged administration of vasopressin and water Am J Physiol 196 451 1959
62 Cannon J F Diabetes insipidus Clinical and experimental studies with consideration of genetic relationships A M A Arch Int Med 96 215 1955
63 West J R and Kramer J G Nephrogenic diabetes insipidus Pediatrics 15 424 1955
64 Allen G Personal communication
65 Crawford J D and Kennedy G C Chlorothiazide in diabetes insipidus Nature London 183 891 1959

# Chapter 41

# Glycinuria

*James B. Wyngaarden*

In 1957 DeVries, Kochwa, Lazebnik, Frank, and Djaldetti [1] reported a family in which excessive urinary excretion of glycine was found in association with nephrolithiasis. The glycinuria appeared to be due to a selective renal tubular defect of glycine reabsorption and was not accompanied by unusual loss of other amino acid, phosphate, or glucose. A kidney stone obtained from one patient was composed chiefly of calcium oxalate but also contained a small amount of extractable glycine. To date this is the only report of what is probably a very rare disease.

## CLINICAL SYNDROME IN THE INDEX CASE

This disorder was first recognized in a 20-year-old woman whose initial urologic symptoms occurred at 2 years of age, when she had fever associated with pus in the urine. The first of a series of attacks of renal colic occurred 2 years later. Renal lithiasis was recognized roentgenographically at age 6. One pyohydronephrotic kidney was removed at 11, and 8 years later a calculus developed in the remaining kidney. Examination then showed many calcium oxalate crystals in the urine, but no infection. When the patient was 19, pyelotomy was performed and the stone was removed. On a high fluid intake the urine subsequently showed a trace of albumin but no cells, casts, or crystals.

## METABOLIC STUDIES IN GLYCINURIC SUBJECTS

The urine of the patient was found to contain unusual amounts of glycine but normal quantities of other amino acids. Initial studies were performed by two-dimensional paper chromatography. Quantitative estimations of the amount of glycine in the urine were obtained by a microbiologic assay. Six members of the patient's family were also

examined and glycine excretion was abnormally high in three Two family members also gave a history of renal colic Data on these family members are shown in Table 41 1 It will be noted that abnormal glycine excretion was found in a total of four individuals all women representing three successive generations

TABLE 41 1 DATA ON GLYCINURIC FAMILY

| Family members | Age yr | History | | Glycine in urine mg/24 hr* | Glycine in serum† |
|---|---|---|---|---|---|
| | | Renal colic | Stones passed or diagnosed (including x ray) | | |
| Propositus | 20 | + | + | 911 | Normal |
| Mother | 48 | − | − | 536 | Normal |
| Father | 52 | − | − | 195 | |
| Sister | 22 | + | + | 632 | Normal |
| Grandmother (maternal side) | 75 | + | − | 593 | |
| Uncle (maternal side) | 55 | − | − | Normal† | |
| Daughter of sister | 2 | − | − | 1 0 | |

* Microbiologic assay
† Two-dimensional chromatography
SOURCE DeVries et al [1]

TABLE 41 2 INTRAVENOUS GLYCINE LOADING STUDY

| Subject | Urinary free glycine load, mg | |
|---|---|---|
| | 0 min | 200 min |
| Normal | 240 | 334 |
| Normal | 85 | 340 |
| Glycinuric | 850 | 1 240 |

Each subject received 1 gm glycine per 15 lb body weight in 70 ml saline solution within 20 min
SOURCE DeVries et al [1]

The serum level of glycine in three glycinuric individuals was normal as judged by a semiquantitative paper chromatographic evaluation Consequently it was believed that the glycinuria could not be attributed to overflow secondary to hyperglycinemia A disturbance of renal tubular reabsorption of glycine was therefore postulated Studies of glycine excretion following intravenous infusion of glycine showed a significantly greater urinary loss of glycine in the original patient than in two normal subjects similarly investigated (Table 41 2) The urinary

glycine excretion of the glycinuric subject was not influenced by 3 days of ingestion of a diet containing only 30 gm protein per day

The stone which was obtained was composed mainly of calcium oxalate but free glycine was extractable from the stone at room temperature with 1 N HCl and proved to be of the order of 0 5 per cent of the dry weight of the stone No glycine was found in eight random oxalate stones obtained from other patients The possibility of an abnormal degree of conversion of glycine to oxalate via glyoxylate (cf Chap 14) was considered but the urine contained only 18 to 40 mg oxalate per day on nine consecutive days and glyoxylic acid could not be demonstrated in her urine

Additional studies on two members of this family revealed normal calcium excretions on a regular diet normal responses to intravenous calcium infusions and normal phosphorus clearances No glycosuria or abnormal xanthinuria was detected in the propositus Except for the maternal grandmother no family members showed evidence of decreased bone density by roentgenographic study Thus these subjects appeared to exhibit a disorder different from those reported by Evered [2] whose patients showed glycinuria hypophosphatemia hyperphosphaturia and prolinuria associated with osteomalacia (cf Chaps 36 and 37)

## SOME ASPECTS OF NORMAL GLYCINE METABOLISM

Glycine is abundantly present in the diet and is found in all proteins It is a nonessential amino acid and is readily synthesized by the majority of living organisms *The principal source of nondietary glycine in the* animal body is serine [3 4] which can be readily transformed into glycine by loss of its $\beta$ carbon as formyl A second source is by transamination of glyoxylic acid from glutamic acid Glycine can also be generated by catabolism of sarcosine or of ingested threonine

Glycine is an important compound in many biosynthetic pathways In addition to its role in protein synthesis and its ready transformation into intermediates of carbohydrate metabolism glycine is directly involved as a precursor of purines porphyrins glyoxylic and oxalic acids serine glutathione sarcosine creatine and glycocholic acid It is found in urine as the benzoyl peptide hippuric acid and also forms conjugates with certain steroids salicylates and Bromsulfalein

Estimates of the glycine pool have been made in rats and more recently in man Arnstein and Neuberger [5] estimated that the readily available glycine pool of the rat is of the order of 100 mg per kg body weight Watts and Crawhall [6] estimated the glycine pool in man to be 80 mg per kg on the basis of measurements of the pecific activity of free urinary glycine following admini tration of glycine $C^{14}$ Gutman Yu Black Yalow and Berson [7] estimated the glycine pool in man to be

83 mg per kg from the ratio of isotope incorporation of glycine 1 $C^{14}$ into urinary uric acid under tracer and load conditions

The glycine pool is not homogeneous Arnstein and Neuberger [5] have evidence for a second glycine pool in rats which is in slow equilibrium with the first or readily available glycine pool Glycine was withdrawn from it in response to large doses of benzoate Benedict Kalinsky, Scarrone, Wertheim and Stetten [8] observed that there are significant differences in labeling of hippurate and the sarcosine moiety of creatine in dystrophic subjects following oral administration of glycine $N^{15}$ They suggest that there are at least two glycine pools in poor equilibrium with one another Also, Watts and Crawhall [6] observed a greater isotopic labeling of the glycine moiety of hippurate than of free urinary glycine in man following oral administration of glycine 1 $C^{13}$ Presumably, the hepatic glycine pool was preferentially labeled under this circumstance

The rate of turnover of glycine has been estimated in several species In the rat and guinea pig the daily biosynthesis of glycine was estimated to be about 2 and 3 gm per kg per day respectively [3, 5] and these values were not significantly influenced by restriction of glycine intake [3] or by vitamin $B^{12}$ or ascorbic acid deficiency, but were reduced in folic acid deficiency [9] Watts and Crawhall [6] estimated the turnover of glycine in man to be only 1 0 gm per kg per day

The plasma levels of glycine normally range from 1 15 to 1 92 mg per 100 ml as determined by resin column chromatography [2 10] and 1 47 to 2 83 mg per 100 ml as determined chemically [11] By the latter method the values for whole blood are higher (1 76 to 3 02 mg per 100 ml) because of the greater concentration of glycine in the red cell (1 64 to 3 73 mg per 100 ml) Variations of concentration of glycine in plasma blood or red cells have been reported in disease [11] Plasma glycine levels decrease significantly (20 to 32 per cent) following oral ingestion of 10 gm sodium benzoate [12]

## RENAL HANDLING OF GLYCINE

### *Normal Mechanisms*

The normal urinary excretion of free glycine is 70 to 200 mg per day as determined by column chromatographic [2 10] or microbiologic methods [1] A considerably larger amount of glycine is excreted in conjugated form and is released by acid hydrolysis [2 10] The total glycine excretion may normally amount to 800 to 1 125 mg per day

Glycine clearance has been estimated in only a few subjects It ranges from 1 5 to 5 7 ml per min [?] Presumably glycine is freely filtered at the glomerulus and quite efficiently reabsorbed in the renal tubules The mechanism by which it is reabsorbed is not well understood Such studies as are available have been performed chiefly in the dog Pitts s [15] data indicate that glycine and creatine are reabsorbed by a common mecha

nism since as the filtered load of glycine is increased creatine reabsorption decreases and is reduced to zero when the reabsorptive mechanism is saturated with glycine On the other hand elevation of plasma creatine concentration had no significant effect on reabsorption of glycine Thus the reabsorptive mechanism has a much higher affinity for glycine than for creatine Since at sufficient loads alanine and glutamic acid also interfere with creatine reabsorption and since moderate elevations of *plasma glycine significantly depress reabsorption of arginine Pitts subsequently [14] suggested that these four amino acids and creatine are* reabsorbed by a common mechanism It was also suggested that phosphate reabsorption shares a common element with alanine and glycine since elevation of plasma levels of these amino acids depresses phosphate reabsorption [15] Beyer Wright Skeggs Russo and Shaner [16] were unable to confirm Pitts finding that glycine depresses reabsorption of arginine

Arginine and glycine may not be reabsorbed by a common mechanism in man The aminoaciduria of cystinuria involves cystine lysine ornithine and arginine but not glycine [17] whereas certain aminoacidurias involving glycine do not involve arginine [1 ] Indeed the recognition of glycinuria as an isolated reabsorptive defect was interpreted by DeVries and collaborators as evidence that glycine is handled by a tubular reabsorptive mechanism of unique specificity for this amino acid in man While this may be correct an alternative explanation is that a single enzyme normally responsible for glycine alanine and creatine reabsorption (and perhaps of other substances) has selectively lost its ability to bind glycine through a minor structural alteration which is of no consequence for the binding of other substrates Variation in a single amino acid of the peptide chain of the enzyme as in hemoglobins A S and C *[18] might hypothetically have such a consequence It would be of in*terest to know whether the glycinuric subjects of DeVries and collaborators also excreted excessive quantities of creatine

*Renal Mechanism in Glycinuric Patients*

The reabsorptive defect in the glycinuric subjects was of impressive degree The clearance of glycine approximated 70 ml per min in the index case a value perhaps fifteen times normal and larger than the glycine clearance reported by Evered [2] in patients with the adult Fanconi syndrome (41 ml per min) or Hartnup syndrome (29 ml per min) in whom *a generalized aminoaciduria was present In the subjects* with glycinuria the excretion of free glycine ranged from 593 to 1 000 mg per day In subjects with various oligo- or generalized aminoacidurias [2 13 19] the excretion of free glycine has ranged from 220 to 1 010 mg per day Even the largest of these values approximates only 3 per cent of the daily glycine turnover in man It is therefore not surprising that the plasma levels of glycine have been essentially normal in the glycinuric

subjects [1] and also in subjects with adult Fanconi and Hartnup syndromes [2] and Wilson's disease [19]

The relationship between excretion of glycine and formation of calcium oxalate stones in the glycinuric subjects remains obscure Excessive conversion of glycine to oxalate seems quite improbable in view of normal urinary values of oxalate The one stone which has been examined contained glycine which resisted simple elution with water but which was readily extractable in 30 min at room temperature in 1 $N$ HCl This finding should differentiate this type of oxalate stone from those commonly encountered in nonglycinuric subjects

## GENETICS

Glycinuria is inherited as a dominant condition possibly sex limited, in the only available pedigree (Fig 41 1) The pedigree is too small to

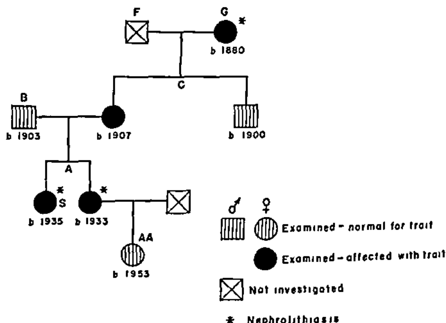



Fig 41 1 Pedigree of glycinuria (*Redrawn from DeVries et al [1]*)

establish sex limitation with certainty or to determine whether the trait is transmitted by an autosomal or sex chromosome

## SUMMARY

1 Glycinuria is a rare disorder which has been recognized in four female members of three generations of a single family It appears to be due to a selective renal tubular defect of glycine reabsorption

2 Three of the glycinuric subjects had nephrolithiasis The only stone analyzed was composed chiefly of calcium oxalate but it also contained a small amount of extractable glycine The excretion of oxalate in the urine was normal

3 The pedigree of the glycinuric family suggests that the disorder is transmitted as a dominant character possibly sex limited

## BIBLIOGRAPHY


1 DeVries A Kochwa S Lazebnik J Frank M and Djaldetti M Glycinuria a hereditary disorder associated with nephrolithiasis Am J Med 23 408 1957
2 Evered D F The excretion of amino acids by the human A quantitative study with ion exchange chromatography Biochem J 62 416 1956
3 Arnstein H R V and Neuberger A The synthesis of glycine and serine by the rat Biochem J 55 271 1953
4 Arnstein H R V and Keglević D A comparison of alanine and glucose as precursors of serine and glycine Biochem J 62 199 1956
5 Arnstein H R V and Neuberger A Hippuric acid synthesis in the rat Biochem J 50 154 1951
6 Watts R W E and Crawhall J C The free glycine metabolic pool in man Biochem J 73 277 1959
7 Gutman A B Yü T F Black H Yalow R S and Berson S A Incorporation of glycine-1 $C^{1}$ glycine 2-$C^{1}$ and glycine-$N^{+}$ into uric acid in normal and gouty subjects Am J Med 25 917 1958
8 Benedict J D Kalinsky H J Scarrone L A Wertheim A and Stetten D Jr The origin of urinary creatine in progressive muscular dystrophy J Clin Invest 34 141 1955
9 Arnstein H R V and Stankovic V The effect of certain vitamin deficiencies on glycine biosynthesis Biochem J 62 190 1956
10 Stein W H A chromatographic investigation of the amino acid constituents of normal urine J Biol Chem 201 45 1953
11 DeVries A and Alexander B Studies on amino acid metabolism II Blood glycine and total amino acids in various pathological conditions with observations on the effects of intravenously administered glycine J Clin Invest 27 655 1948
12 DeVries A and Alexander B Studies on amino acid metabolism III Plasma glycine concentration and hippuric acid formation following the ingestion of benzoate J Clin Invest 27 665 1948
13 Pitts R F A renal reabsorptive mechanism in the dog common to glycine and creatine Am J Physiol 140 156 1943
14 Pitts R F A comparison of the renal reabsorptive processes for several amino acids Am J Physiol 140 535 1943
15 Ayer J L Scheiss W A and Pitts R F Independence of phosphate reabsorption and glomerular filtration in the dog Am J Physiol 151 168 1947
16 Beyer K H Wright L D Skeggs H R Russo H F and Shaner G A Renal clearances of essential amino acids their competition for reabsorption by the renal tubules Am J Physiol 151 202 1947
17 Stein W H Excretion of amino acids in cystinuria Proc Soc Exper Biol & Med 78 705 1951
18 Hunt J A and Ingram V M Allelomorphism and the chemical differences of the human haemoglobins A S and C Nature London 181 1062 1958
19 Stein W Bean A G and Moore S The amino acid content of the blood and urine in Wilson s disease J Clin Invest 33 410 1954

# Chapter 42

## Cystinuria*

W. Eugene Knox

✓ Cystinuria was one of the four diseases singled out by Garrod in 1908 [1] as the "inborn errors of metabolism." He postulated that cystinuria was due to a block in oxidation of cystine to sulfate. Increasing understanding of this disease has necessitated revision of Garrod's original concept and expansion of the "inborn errors" to include not only hereditary defects of dissimulative and synthesizing enzymes but also defects of certain other functional systems, such as renal tubular transport mechanisms.

Cystinuria is now known to be a hereditary anomaly of renal function in which there is impaired renal tubular reabsorption of cystine, lysine, arginine, and ornithine. These amino acids, two of them essential to the body, are excreted in the urine in abnormal amounts throughout life. Only the excretion of the least soluble one, cystine, was recognized until recent times, and both the name and the clinical importance of the condition are entirely referable to this one amino acid. The only clinical consequence appears to be the frequent formation of urinary calculi composed of almost pure cystine. Some of the individuals heterozygous for the trait of cystinuria also excrete more than the normal amount of cystine, but the amount is not large, and these individuals only rarely form stones.

Few of the aspects of cystinuria now remain unknown. Perhaps the major deficiency is proof that the two hereditary types, the recessive and incompletely recessive forms of the disease, are caused by allelic genes. But much remains to be done on the practical side in the recognition and treatment of cystinuric patients. The principles are well established and if effectively utilized could probably prevent calculus formation and its complications in most homozygotes.

* This work was supported in part by grant AT(30-1)-901 from the U.S. Atomic Energy Commission and by grant A-567 from the Public Health Service.

There is much to be learned from the multitudinous research efforts on cystinuria in the past The approach to be employed in this chapter is historical in order that from a vantage point of contemporary concepts the tortuous development of knowledge of the disease can be viewed in illuminating perspective

## EARLY HISTORY AND DEFINITION

### *Initial Recognition*

Cystine and cystine stones were recognized long before cystinuria Indeed the cystine with which many studies began was provided by the



Fig 42 1 Cystine stones from bladder (*left* 50 2 gm) from kidney (*middle*) and from ureter (*right* 40 7 gm) of patients originally reported by Morner [J] Although their appearance may be distinctive many such stones cannot be identified without chemical analysis (*By permission of A Renander* [J])

patients themselves upon whom the supply of this essential material was dependent for nearly 100 years In 1904 [2] and even in 1936 [3] cystine metabolism was tested by giving to an individual cystine from a stone which he had formed

Wollaston who 13 years earlier had described five different types of urinary calculi and demonstrated that extrusions from gouty tophi were identical with one of these added a sixth type in a report to the Royal Society of London in 1810 On Cystic Oxide a New Species of Urinary Calculus [4] (see Fig 42 1) Two examples of bladder stones had come into his hands Wollaston considered the substance to be an oxide because of its tendency to unite with both acids and alkalies Because of its occurrence in the bladder (Greek *kystis*) he gave it the name *cystic oxide*

The name became a focus in the first debates about the new disease Stones do develop in the bladder in cystinuria [J] especially in young

people It was assumed that cystine stones were, therefore, formed by the bladder until in 1818 Marcet found three patients (two at autopsy) with renal stones of cystine Berzelius observed that the substance was not an oxide and suggested the name *cystin* Garrod observed in his third Croonian lecture [1] Of this new name which has since been universally adopted Civiale wrote in 1838, that although it corrected an *error of chemistry it perpetuated an error of physiology*, for cystine is excreted by the kidneys and does not have its origin in the bladder'

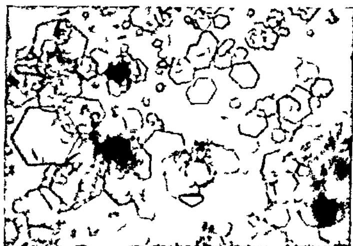


Fig 42-2 Typical microscopic hexagonal plates of cystine in sediment of cystinuric urine Angles are each very nearly 120 despite irregular shape (*By permission of A Renander* [5])

The renal origin of cystine was established when Prout in 1820 and Strohmeyer in 1824 (cited in [3]) recognized the same hexagonal platelets of cystine in the urinary sediment which Wollaston had formed from *dissolved stones The identification of these crystals through a microscope* has remained until recent times the primary means of diagnosis of cystinuria (Fig 42 2) The collection and weighing of these crystals was first done in 1855 by Toel (cited in [5]) who found a cystine excretion of 1 33 to 1 50 gm per day in a cystinuric patient This procedure also became the basic method for the study of cystine metabolism until it was replaced after 1900 by Folin s urinary sulfur fractionations

The slowly accumulating information from sporadic cases of cystinuria *was harvested and first given form by Niemann in 1876 [6]* He tabulated in chronologic order and discussed in addition to his own patient, 52 case which he found in the medical literature

*Diagnostic Criteria*

Nearly all the cases in Niemann s collection had been diagnosed on the basis of urinary stones of cystine although several had been recognized

by cystine crystalluria He made careful drawings and crystallographic studies of these transparent shiny hexagonal leaflets seen with a microscope in the sediment of urine which had been allowed to stand for a few hours (Fig 42 3) The identification of cystine rested primarily upon

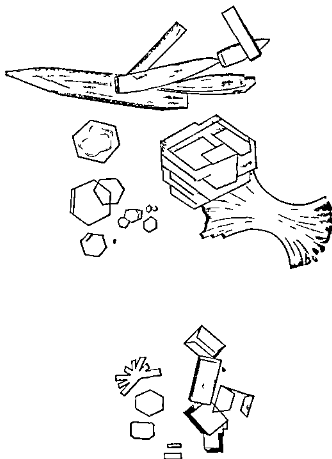


Fig 42-3 Niemann s engraving of crystal forms of cystine Only microscopic hexagonal plates form spontaneously in urine (middle left) Recrystallization by evaporation after solution with ammonia gives plates and prisms or prismatic needles grouped above and to right of hexagonal plates Recrystallization from acetic acid (*below*) rarely gives hexagonal plates but numerous prismatic forms (From *Niemann* [6])

Wollaston s criteria the solubility of the crystals in ammonia and insolubility in acetic acid and when strong HCl was added to the dry crystals under the microscope the rapid growth of stellate clusters of delicate prisms consisting of the water soluble cystine hydrochloride Chemical analysis depended largely upon the sulfur reaction the

formation of a precipitate of lead sulfide when urine or a cystine solution was heated with KOH in the presence of lead acetate He recognized that cystine could be present in the urine in abnormal amounts as indicated by the lead sulfide precipitate without the presence of stone or crystals

By Niemann s criteria, a diagnosis of cystinuria suggested by a stone or crystals was confirmed by the identification of cystine through the strong sulfur reaction given by the solid material Additional confirmatory evidence was the smell of hydrogen sulfide when the urine was allowed to stand long in hot weather, or the foul smell due to cadaverine and putrescine formed by bacterial action on the other amino acids now known to be present in these urines in abnormal amounts Since the methods for detecting cystine depended upon precipitation from supersaturated solutions it is not surprising that Niemann was cautious about excluding the diagnosis of cystinuria He noted that cystine crystals are sometimes absent from the urine of individuals with a known cystine stone that cystine crystalluria is sometimes present without stone and that sometimes stones of other chemical composition are found in known cystinuric patients The latter are now known to develop because of the urinary infections set up by the original stones

*Patterns of Occurrence*

Both males and females are affected with cystinuria and males predominate Niemann s collection of 53 patients was comprised of 38 males 14 females and 1 patient of unstated sex A similar predominance of males though slightly less marked has characterized all subsequent collections of cases [7–9] No significant sex difference however, occurred in a later group of cases diagnosed by chemical examination [10]

Niemann believed that cystinuria is present from birth The youngest patient seen up to that time was 2 years old Frequencies of stone formation were similar in the successive decades of life [6] and no stones were reported after age 50 Since then a cystine stone has been passed spontaneously by a child of 9 months [11] and several cases are now on record of stone formation in older persons including a woman of 87 [12] In one patient stone formation occurred at age 42 and recurred at age 81 [13]

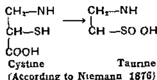
Niemann commented on the familial disposition of cystinuria The fourth and fifth cases of Marcet (cited by Niemann) in 1818 were two brothers about 30 years old Four other instances in which two siblings were affected had been reported and in one of these the mother had also excreted abnormal amounts of cystine but did not have stones or crystalluria It is interesting that the brother of Niemann s patient who might well have had the same genotype excreted abnormal amounts of cystine by chemical test the mother and maternal uncle of the patient were normal in this regard

*Possible Causes*

His large collection of cases put Niemann on firm ground to evaluate the alleged constitutional or environmental causes of the disease There was no association with occupation social standing housing or nutrition or with scrofula anemia or rheumatism

In 1868 Marowsky [14] from observations on a patient with cystinuria who also had chronic acholia and liver disease theorized that cystinuria is caused by liver disease He believed that in cystinuria the usual taurine-secreting activity of the liver is suppressed and replaced by vicarious secretion of the sulfur containing taurine through the kidney in the form of cystine Niemann however found no evidence for the appearance in the urine of other fragments of the bile acids and Lewis [15] later cited analyses showing no deficiency of taurocholic acid in the bile of cystinuric patients

Niemann revamped Marowsky s hypothesis into the form later to be known as an arrest of metabolism a concept from which Garrod developed his fertile idea of the inborn errors of metabolism Instead of taurine s being converted to cystine as Marowsky implied the formulas of the substance as then known



(According to Niemann 1876)

indicated to Niemann the rever e relation (the correct formulation does not alter this conclusion) that taurine is the natural oxidation product of cystine His own measurements disclo ed a low sulfate excretion in cystinuria He failed to add the now obvious suggestion that a failure of cystine oxidation would cause the low urinary sulfate but the pathway of cystine oxidation through taurine to sulfate was not yet known

*Treatment*

No treatment was used for Niemann s patient [6] He reviewed earlier attempts at treatment and suggested that alkalinization of the urine might di solve the cystine Magendie is credited with the same suggestion plus restriction of protein (cited in [5]) On one occasion Niemann s patient *excreted an alkaline urine containing cystine crystals* This observation in addition to the fear of precipitating phosphate with alkali and so enlarging the stones already present restrained him from trying alkali therapy Only surgical treatment was offered when necessary and that sometimes tardily by modern standards

*Definitions*

Niemann s success in defining cystinuria, achieved largely by the use of logic to divest the known cases of their adventitious trappings can be measured by comparing the conclusions with the definition of cystinuria written in 1955 by Dent and Senior [16]

> It is a condition presumed to be present from birth and characterized by the excretion in the urine of large quantities of cystine (in the adult about 1 gm in 24 hours) lysine arginine and ornithine The condition is often present in the patient s siblings and may result in the formation of stones composed almost entirely of cystine Apart from the possibility of kidney damage and the other complications resulting from stone formation the patients enjoy good health and are clinically indistinguishable from normal

Niemann s definition agrees with this modern one in every detail with the exception only of the abnormal amounts of lysine arginine, and ornithine in the urine of these patients

## CLINICAL AND BIOCHEMICAL NATURE OF CYSTINURIA

*Clinical Reports*

After Niemann s review little could be added of clinical importance except validation of his definition of the disease until additional under standing was available This was long in coming Ebstein [17] 8 years later added 10 cases, and in 1900 Simon [18] brought the total to 103 cases and added 4 of his own and 1 from the Norwegian literature One of his cases was a Negro the only one reported to have cystinuria These cases and others have been repeatedly reviewed [7, 19–21] Sum Schick counted 180 cases by 1929 [22] In the 10-year period between 1921 and 1932 Lewis [15] counted 71 newly reported cases Morrison [11] listed 75 cases studied radiographically between 1920 and 1940 Renander s estimate that published cases by 1940 only slightly exceeded 200 was conservative [5]

Some cases have been of unusual interest Southam [23] removed a cystine stone from a woman who 14 years postoperatively still excreted cystine The mother of this patient had been operated on 24 years earlier by Southam s father also for cystine stone and she continued to excrete cystine for 8 years The chronicity of the disease was further documented by a metabolic study in 1923 by Looney, Berglund and Graves [24] of a patient studied 17 years earlier by Alsberg and Folin [25]

A dignified rivalry to report the largest stone can be discerned in the clinical literature Chaos governed the contest since the participants frequently confused weights in *grams* with *grains* The record, attested by an independent weighing 27 years later when the stone reposed in the Museum of the Royal College of Surgeons in England was long held

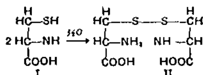
by a vesical calculus weighing 68 04 gm and described by Harrison in 1849 [26] Link found a stone of 86 gm in 1912 [21] Ancherson in 1914 removed from the bladder of a Norwegian male a cystine stone weighing 102 5 gm the largest that has ever been reported [27]

*Confusion with Cystinosis*

A most notable confusion about the clinical nature of cystinuria was introduced by Abderhalden's description in 1903 of another disease of cystine metabolism [28] Although Abderhalden called the new disease *familial cystine diathesis* the majority of medical writers up to the present time have considered it to be a manifestation of the same hereditary disturbance as cystinuria After the next cases were described in 1926 by Lignac Leiden [29] it was also called *cystinosis cystine storage disease* and *Lignac-deToni Fanconi syndrome* (see Chap 37) The first patient was seen only after death at age 21 months The internal organs were potted with crystalline deposits of cystine Abderhalden demonstrated abnormal amounts of cystine in the urine of two siblings the father (9 2 mg per 100 ml) and the grandfather (14 0 mg per 100 ml) of his patient A reason for additional confusion was that Lignac s first case had ureteral and renal cystine stones (1926) a unique finding in any condition except cystinuria The typical findings of cystinosis all of which distinguish it from cystinuria are retarded growth and development rickets and osteomalacia low serum phosphorus glycosuria generalized aminoaciduria of 10 or more amino acids among which cystine is usually not prominent very rare formation of cystine stones the presence of cystine crystals in the tissues of children but not adults with the disease [30] and its frequently fatal termination

## BIOCHEMISTRY OF CYSTINE

A more precise definition of cystinuria was dependent upon knowledge of the chemical nature of cystine and of its metabolism It would seem that both these aspects were well on the way to solution by 1905 Morner in 1899 [31] had discovered cystine in protein and found hydrolyzed hair to be an unusually rich source The correct chemical formulas of cystine (II) and its reduced form cysteine (I) were established in 1902 by Friedman [32]




Chemical methods to measure urinary neutral sulfur (largely cystine) and organic sulfate were developed The first balance studies on the sulfur

metabolism of a cystinuric person were reported in 1905 by Alsberg and Folin [35]

Loewy and Neuberg [2] reported in 1904 that hair and stone cystine are metabolized differently and are different chemical substances Alsberg and Folin [35] promptly reported their own and other evidence against this conclusion They were unable to verify such a difference (which hinged on a difference of crystal form), but as late as 1927 after the analysis of the 78 gm of stones from Tennant s patient [33] this error was repeated [34] There was further confusion when it was observed that an increase in food protein produced an increase of cystine in the urine but when the food protein was hydrolyzed outside the body and the isolated cystine was given to the patient, that cystine was oxidized to sulfate Cystine itself even cystine isolated from the patient's own urine [35] caused no extra cystine to be excreted Garrod [36] expressed the pessimism of the time when he wrote

> The study of the metabolic peculiarities of cystinurics has yielded results which are very difficult of explanation and the more the problem is investigated the more remote seem to be the chances of its satisfactory elucidation Thus in every case tested except that of Loewy and Neuberg in which cystine has been given by mouth it has been completely destroyed although the patients were all the time excreting cystine as such

During the same period other observations were made from which the modern understanding of cystinuria could have been deduced But like a hank of string with the key loop in sight, several pulls at other loops resulted in a snarl which took nearly 50 years to untangle

### CYSTINURIA AS AN ' INBORN ERROR OF METABOLISM"

Garrod announced his brilliant concept of the inborn errors of metabolism at the height of the confusion about cystine metabolism [1] According to Garrod s thesis a hereditary condition characterized by the accumulation of an abnormal metabolite resulted from the hereditary lack of an enzyme that normally removes that metabolite Here was an illuminating way to regard the accumulation of cystine, which in a normal individual is oxidized to sulfate Cystinuria was included as one of the inborn errors of metabolism This helped to obscure for a generation that a supposedly degradative enzyme in cystinuric subjects was in fact not missing In both normal and cystinuric subjects cystine administered orally as such was oxidized and excreted as sulfate The defect in cystinuria was manifested only if some precursor of cystine such as protein was fed Garrod was not unaware of this paradox but he contented himself with the explanation that the metabolic error was partial and incomplete and that the contradiction would be resolved when more was known of the intermediary steps of sulfur metabolism

There was ample evidence of the hereditary nature of cystinuria Pfeiffer [37] reported four cystinuric children of parents who were first cousins Cohn [38] described a family with a cystinuric mother a normal father and 10 children Six of the children were cystinuric 2 definitely not and 2 untested Two of the children were twins both cystinuric Identical twins both with the disease who also developed stones almost simultaneously, were later described by Kretschmer [7] and in another instance by Harris and Warren [39] Garrod saw that this pattern was *meaningful homozygotes for a rare recessive gene would usually occur* among siblings, rarely in other generations of the same family and more commonly in families where a consanguineous marriage improved the chances that both parents would possess the rare gene He did note however that there was a greater frequency of direct transmission of cystinuria from parent to child than was met in connection with the other metabolic errors he described (i e albinism alcaptonuria and pentosuria) Fifty years later the recognition of a dominant form manifesting cystinuria in the heterozygote confirmed his impression [10]

## METABOLISM OF CYSTINE

In 1935 the biochemist Brand and the urologist Cahill with two young cystinuric patients began to elucidate the metabolic pathway of the sulfur-containing amino acids by feeding experiments The erroneous assumption of a block in cystine metabolism was useful The identification of cystine precursors was as well served by the renal leakage of the extra cystine as it would have been by the supposed metabolic block The new sulfur containing amino acid methionine was promptly identified as a cystine precursor [40] Proteins with high methionine content produced greater cystine excretion than proteins with low methionine content The cystine in the protein was oxidized to sulfate [41] This identification of methionine and not cystine as the principal dietary source of urinary cystine was *confirmed by Lewis [3] and partially resolved the paradox* described earlier The subjects of Brand s experiments formed extra cystine from cysteine and from homocysteine but not from cystine or homocystine [42] Brand postulated that cysteine is a product of the catabolism of methionine and that the error in cystinuria is a failure of the proper utilization of cysteine not a failure of cystine metabolism The final results clearly indicated a pathway of metabolism of sulfur containing amino acids with the surprising sequence of reactions shown in Fig 42-4 [40]

The excretion of cysteine as cystine by cystinuric patients and oxidation of cystine by both normal and cystinuric persons was in accord with what was known The reaction difficult to believe was the shortening of the carbon chain in the conversion of the $\gamma$ thio compound homocysteine to the $\beta$ thio compound cysteine Lewis confirmed the essential

findings for the scheme of Fig 42-4, but he refused to accept the same conclusion

Brand's scheme [43] was confirmed in detail by studies from du Vigneaud's laboratory $S^{35}$ labeled methionine gave rise to labeled cystine but $C^{14}$-labeled methionine did not [44] The shortening of the carbon chain occurs by transfer of the sulfur from the four-carbon compound homocysteine, to a different three carbon compound, serine, to form cysteine made up of the original sulfur but with new carbon atoms The intermediate of this interchange is cystathionine, a combination of both homocysteine and serine in one molecule This compound, too was later shown to give rise to cystine [45]

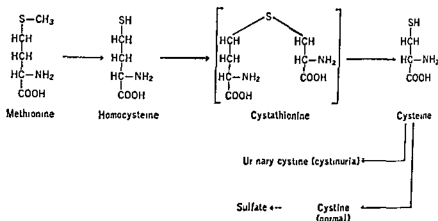



Fig 42-4 Pathway of metabolism of sulfur amino acids established in cystinuric patients by feeding experiments (From *Brand et al* [40]) Cystathionine was discovered later

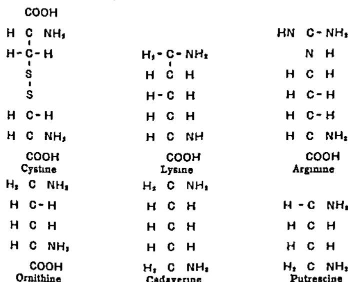
After the pathway of sulfur amino acid metabolism in man was delineated by Brand through his studies of a patient with cystinuria a similar pathway (in reverse direction leading to methionine) was proved for *Neurospora* by the use of several mutants of this species [46]

The studies of Brand and his group still fell short of a full explanation of the disease The enzymic apparatus for cystine degradation by the cystinuric patient is obviously intact since cystine given as such is excreted as sulfate Any amount given orally can be oxidized by the liver but if cystine or cysteine is given parenterally to dogs or rabbits [47] or to a cystinuric patient [48] it is excreted by the kidney as cystine Failure of tubular resorption was later implicated quite directly by the fact that the level of cystine in the blood of cystinuric patients is not elevated [49] This eliminated the possibility of a renal overflow mechanism although it was apparently not appreciated by the authors at the time The meaning was not lost to all however for in 1934 Rabinowitch suggested that the mechanism of cystinuria is analogous to that of glycosuria

in renal glycosuria (which he thought was a result of an undue 'permeability' of the kidney [50]) There was left unexplained then only why the kidneys are presented with a greater load of cystine for excretion when a precursor is fed than when cystine is fed as such this question has still not been answered [51]

## AMINOACIDURIA OF CYSTINURIA

An earlier series of experiments might have led to the recognition of the other amino acids (lysine arginine and ornithine) that are present in abnormal amounts in cystinuric urine




Ellinger [52] in 1898 found that bacteria would decarboxylate lysine and ornithine to form the foul smelling ptomaines or diamines cadaverine and putrescine These substances had been isolated from the urine of many [18, 25] but not all cystinuric patients The original patient studied in 1889 [53 54] had cystitis and successive investigators over several years found the amounts of diamines increasing in pace with her urinary infection [55] Recently Harris et al [10] reported a cystinuric patient who was exceptional among their patients in excreting no lysine Upon closer examination they found a urinary tract infection and further analysis of the urine revealed the missing lysine present as cadaverine

It seems now almost inconceivable that the work of Alsberg and Folin in 1905 [25] and its extension by Wolf and Shaffer in 1908 [48] did not quickly solve the problem of cystinuria Alsberg and Folin tested the theory of defective hydrolysis of dietary protein by measuring the 'undetermined nitrogen' fraction of urine i e that part of the total

findings for the scheme of Fig 42-4, but he refused to accept the same conclusion

Brand's scheme [43] was confirmed in detail by studies from du Vigneaud's laboratory $S^{35}$ labeled methionine gave rise to labeled cystine, but $C^{14}$-labeled methionine did not [44] The shortening of the carbon chain occurs by transfer of the sulfur from the four carbon compound, homocysteine to a different three carbon compound, serine to form cysteine made up of the original sulfur but with new carbon atoms The intermediate of this interchange is cystathionine, a combination of both homocysteine and serine in one molecule This compound too, was later shown to give rise to cystine [45]

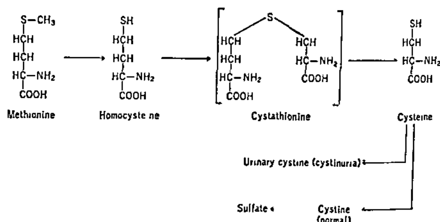



Fig 42-4 Pathway of metabolism of sulfur amino acids established in cystinuric patients by feeding experiments (From *Brand et al* [40]) Cystathionine was discovered later

After the pathway of sulfur amino acid metabolism in man was delineated by Brand through his studies of a patient with cystinuria, a similar pathway (in reverse direction leading to methionine) was proved for *Neurospora* by the use of several mutants of this species [46]

The studies of Brand and his group still fell short of a full explanation of the disease The enzymic apparatus for cystine degradation by the cystinuric patient is obviously intact since cystine given as such is excreted as sulfate Any amount given orally can be oxidized by the liver but if cystine or cysteine is given parenterally to dogs or rabbits [47] or to a cystinuric patient [48] it is excreted by the kidney as cystine Failure of tubular resorption was later implicated quite directly by the fact that the level of cystine in the blood of cystinuric patients is not elevated [49] This eliminated the possibility of a renal overflow mechanism although it was apparently not appreciated by the authors at the time The meaning was not lost to all however for in 1934 Rabinowitch suggested that the mechanism of cystinuria is analogous to that of glycosuria

The project languished and the dogs were dispersed sometime before Brand s death in 1953, and before the new concept of the renal tubular mechanism in human cystinuria could be tested on cystinuric dogs Metabolic studies nearly duplicated the findings from contemporaneous human studies Cystine accounted for 13 to 17 per cent of the total urinary sulfur in the cystinuric dogs [62 64] compared with about 20

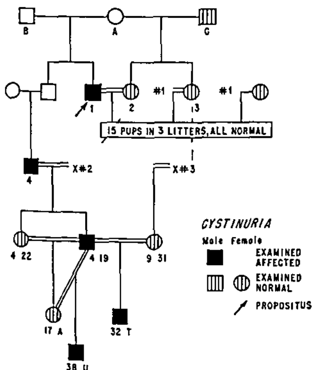



Fig 42-5 Known pedigree information of cystinuria in Irish terriers Original numbering system is retained Litters were undoubtedly larger A total of more than 300 descendents of dogs 1 and 4 were tested and 1 cystinuric animals all males were found (From *B and et al* [62] and *W E Hess and M X Sullivan* [63])

to 30 per cent in the human being with cystinuria [3] Excess cystine was excreted when extra protein methionine or cysteine was fed The feeding of cystine did not result in extra cystine excretion [65]

It remains to be shown whether the same discrete renal transport system under hereditary control is affected in the cystinuria of both men and dogs It was in normal dogs that the competition between arginine and lysine for renal tubular excretion was first demonstrated [65] This finding was initially the basis for the belief that the excretion of the several amino acids in man could be due to a deficiency of a single renal

nitrogen left after subtraction of that determined independently in urea ammonia creatinine, uric acid and cystine Their patient excreted abnormally large amounts of the undetermined nitrogenous substances as well as cystine during starvation They concluded that these were produced by 'endogenous metabolism' but both fractions were also increased in proportion to the protein in the diet and therefore were also produced by 'exogenous metabolism "

Four years later Wolf and Shaffer [48] confirmed the results of Alsberg and Folin in detail and emphasized that there is an abnormal excretion of undetermined nitrogen" in cystinuria and that this is at least in part due to other amino acids Somehow, this was forgotten Later isolations of crystalline lysine derivatives from cystinuric urine by Ackermann and Kutscher in 1911 [55a] and by Hoppe-Seyler in 1933 [56] passed almost unnoticed and the observations were not corroborated Kutscher obtained amounts equivalent to 0 1 gm lysine per liter of urine which was grossly more than could be present in normal urine The discovery and identification of three other amino acids besides cystine in cystinuric urine 40 years later was a major forward step in the understanding of this disease

## CYSTINURIA IN ANIMALS

The sporadic occurrence of urinary cystine stones in dogs was reported in 1823 by Lassaigne [57] in 1861 by Gross [58] and by Lentz in a male dachshund in 1921 [59] The stone was always recognized after the death of the animal and no metabolic studies were made The tendency to form cystine stones was not associated with a single breed, as is the frequent development of urate stones in the Dalmatian coach hound In 1935 Morris [60] found that an Irish terrier suffering from urinary retention had cystine crystalluria At operation several vesicular and urethral calculi of cystine were successfully removed [60] Brand and Cahill joined the study made the necessary chemical measurements to identify the disease and arranged a breeding program to investigate the genetics of the condition

Through the American Kennel Club registrations of the sire and dam of the first cystinuric dog four related dogs were found to begin a breeding program One a cousin of the proband also had cystinuria [61] Twenty five unrelated animals of the same breed were not cystinuric Three hundred dogs of the cystinuric strain were raised and 12 cystinuric animals were identified All affected animals were males [61 62] The original cystinuric male produced litters with an unrelated female and with two half sisters None of the 15 pups was cystinuric [64] No genetic analysis of the results was ever made but the few published pedigrees resembled or at least did not exclude sex linked inheritance [62 63] one quite different from the recessive pattern seen in man (Fig 42 5)

excretions about ten times the normal level [76] Equally powerful and even more convenient methods of amino acid analysis soon confirmed these new abnormalities Column chromatography of the urinary amino acids in six cystinuric patients all with histories of cystine stone formation showed that a definite pattern of aminoaciduria was characteristic of this disease [77] In addition to the cystine lysine and arginine already identified ornithine was also found in abnormal amounts The amounts of the remaining amino acids were not different from normal

The first explanation of the large amounts of new amino acids found in the urine of cystinuria was progressive renal damage producing first the leakage of these few amino acids and later perhaps the generalized aminoaciduria seen in the Fanconi syndrome [76] However long clinical experience belied this A nonspecific renal damage would not show such specific leakages

*Renal Physiology and Aminoaciduria*

The possibility that a discrete renal tubular transport mechanism with enzyme like specificity could be defective in cystinuria and permit the specific aminoaciduria was first appreciated in 1951 It was known that arginine and lysine compete for the same tubular reabsorptive mechanism in the kidney of the dog each lowering the $T_m$ of the other [6a] Dent and Rose [78] suggested that the reabsorptive mechanism was missing in cystinuria The first evidence to substantiate this was an elaboration of an old finding by Brown and Lewis in 1937 [49] that the concentration of cystine in the blood of a cystinuric patient is not elevated In 10 normal and 9 cystinuric individuals the concentration of cystine in the plasma averaged 0 82 and 0 69 mg per 100 ml respectively [ 9] It was argued that if cystinuria was the result of a renal defect the plasma level would be normal or low as it appeared to be but that if it was the result of a blocked cystine metabolism the plasma level should be elevated Evidence was presented that the levels in blood are not above normal for the other amino acids also excreted in cystinuria [80] Stein and Moore [81] established that high plasma concentrations of amino acids could not account for the excretions since the levels of lysine and ornithine were normal and of cystine and arginine low in their patient

The crucial evidence for a specific renal mechanism was obtained from the renal clearance of the amino acids In a classic study by Dent Senior and Walshe [81] cystine clearance in normal individuals was of the order of 4 ml per min whereas in two cystinuric patients (both with considerable renal damage from stone formation and therefore probably with reduced glomerular filtration rates) the cystine clearances were approximately 100 ml per min i e they approached the glomerular filtration rate Moreover in a person known to be heterozygous for cystinuria a definitely elevated cystine clearance (14 ml per min) was found

transport system Opportunities to make observations on such dogs are apparently not rare Krabbe [66] reported that 3 per cent of the urinary calculi removed from dogs in Holland and Denmark are cystine In England they accounted for 18 per cent, according to White [67] and were the stones which most commonly caused urethral impaction Crane and Turner [68] found cystine and lysine predominating in the urine of a Labrador retriever who had two episodes of cystine vesical calculi with urethral impaction No abnormal quantities of arginine or ornithine were found Study of the plasma was inconclusive This is the urinary pattern expected in the heterozygous form of incompletely recessive cystinuria in man (see below)

A second species of animal with cystinuria is the blotched genet a wild cat from Kenya In a survey by paper chromatography of the amino acids excreted by different animals at a zoo, Datta and Harris [69] found that every blotched genet was cystinuric The condition was present in a number of unrelated members of the species so it was not just a familial condition *within* the species, but a species characteristic

*The cystinuria of the Kenya genet differs from that in man in that* high concentrations of other amino acids are not excreted Dent and Senior [16] also established that the cystinuria occurred by a renal mechanism i e , a deficiency of tubular reabsorption Strangely enough the concentration of cystine in the genet urine is 1 to 2 mg per ml *in true solution* that is about four times its solubility in human urine Yet the genets do not form urinary cystine stones The failure of cystine to precipitate from these urines raises again the possibility of a cystine-containing complex existing in cystinuric urines of man [40, 70, 71] and dog [63] although the existence of such a complex has been emphatically denied [3] No additional cystine lysine or arginine is found in human cystinuric urine after hydrolysis [72] The early belief in such a cystine complex may have arisen from a theory that when cystine is absorbed as a peptide because of faulty digestion it escapes oxidation and produces cystinuria [73] No stable complex of cystine was found in genet urine The unusually high urea concentration of 10 to 20 gm per 100 ml in genet urine and the presence of other salts and amino acids [74] might affect the solubility of cystine

Recently cystine urinary stones have been discovered in a third animal species the mink [75]

## RENAL TUBULE AND CYSTINURIA

### *Pattern of Aminoaciduria*

In 1947 microbiologic determinations of the individual amino acids in the urine of a cystinuric girl (compared with analyses in seven female controls) showed a high cystine excretion but also arginine and lysine

excretions about ten times the normal level [76] Equally powerful and even more convenient methods of amino acid analysis soon confirmed these new abnormalities Column chromatography of the urinary amino acids in six cystinuric patients, all with histories of cystine stone formation showed that a definite pattern of aminoaciduria was characteristic of this disease [77] In addition to the cystine lysine and arginine already identified ornithine was also found in abnormal amounts The amounts of the remaining amino acids were not different from normal

The first explanation of the large amounts of new amino acids found in the urine of cystinuria was progressive renal damage producing first the leakage of these few amino acids and later perhaps the generalized aminoaciduria seen in the Fanconi syndrome [76] However, long clinical experience belied this A nonspecific renal damage would not show such specific leakages

*Renal Physiology and Aminoaciduria*

The possibility that a discrete renal tubular transport mechanism with enzyme-like specificity could be defective in cystinuria and permit the specific aminoaciduria was first appreciated in 1951 It was known that arginine and lysine compete for the same tubular reabsorptive mechanism in the kidney of the dog each lowering the $T_m$ of the other [6o] Dent and Rose [78] suggested that the reabsorptive mechanism was missing in cystinuria The first evidence to substantiate this was an elaboration of an old finding by Brown and Lewis in 1937 [49] that the concentration of cystine in the blood of a cystinuric patient is not elevated In 10 normal and 9 cystinuric individuals the concentration of cystine in the plasma averaged 0 82 and 0 69 mg per 100 ml, respectively [79] It was argued that if cystinuria was the result of a renal defect the plasma level would be normal or low as it appeared to be but that if it was the result of a blocked cystine metabolism the plasma level should be elevated Evidence was presented that the levels in blood are not above normal for the other amino acids also excreted in cystinuria [80] Stein and Moore [81] established that high plasma concentrations of amino acids could not account for the excretions since the levels of lysine and ornithine were normal and of cystine and arginine low in their patient

The crucial evidence for a specific renal mechanism was obtained from the renal clearance of the amino acids In a classic study by Dent Senior and Walshe [51] cystine clearance in normal individuals was of the order of 4 ml per min whereas in two cystinuric patients (both with considerable renal damage from stone formation and therefore probably with reduced glomerular filtration rates) the cystine clearances were approximately 100 ml per min i e they approached the glomerular filtration rate Moreover in a person known to be heterozygous for cystinuria a definitely elevated cystine clearance (14 ml per min) was found

Elevated renal clearances in cystinuric patients of cystine, arginine ornithine, and lysine were then demonstrated by Robson and Rose [82] The clearances of cystine arginine and ornithine were not further increased by the rapid intravenous infusion of 5 gm of L-lysine, because the clearances were already near the glomerular filtration rate In normal individuals and *heterozygotes lysine caused transient increases in the* clearances of the three other amino acids and only of the three that are excreted in cystinuria This is analogous to competitive inhibition of an enzyme

Doolan Harper Hutchin and Alpen [72] also established that the plasma level of lysine was lower than that of normal subjects in four cystinuric patients (2 4 compared with 3 2 mg per 100 ml) and that extra lysine (and arginine) was cleared at a rate similar to that of inulin i e without reabsorption The excretions observed were much too large for leakage of filtrate past a few inactive nephrons A specific defect in renal tubular reabsorption was therefore established

Cystinuria may then be defined as a hereditary inactivity of a specific renal tubular *transport system* which *normally combines* with and reabsorbs the several related dibasic amino acids The inactivity could result from an absence or defect of a specific enzyme-like combining substance in the tubular cells It has been suggested that the enzyme-like combining substance of the transport system fits substrates with two suitably separated amino groups and that this is the structural basis for the common transport of these four dibasic amino acids (see Effect of Genotype on Renal Tubular Transport below) Within the group the affinity for the combining substance ($K_m$ of an enzyme) is less for cystine and lysine than for arginine and ornithine as the genetic studies have indicated (see below)

With the demonstration that a specific renal transport system for certain amino acids is lacking in cystinuria it became possible to ask if this same transport system is operative in other tissues besides the kidney The absorption of amino acids by the intestine and their concentration into cells for example occurs with transport systems apparently similar to those of the kidney tubule There is no positive indication of interference with such systems in cystinuria except in the kidney Only direct tests could rule out such a possibility however and one such observation has been recorded Leukocytes of normal and cystinuric individuals take up cystine and lysine equally efficiently There is no mutual inhibition of uptake by the cells between these two amino acids [83]

## INCIDENCE OF CHEMICAL AND CLINICAL CYSTINURIA

Most surveys [22 84] of which Morner s is the most complete [85] have generally agreed that about 1 per cent of all urinary calculi are

cystine There are no reliable estimates of the incidence of urinary calculi in the general population with which the absolute incidence of cystinuria can be correlated

The incidence of cystine crystalluria has been reported to be 1 case in 15 000 [18] one in 20 000 [86] and four in 35 000 [87] The over all incidence is therefore approximately one in 10 000 urine specimens This is possibly higher than in the general population since undoubtedly only selected (e g hospitalized) populations were tested

The apparent incidence of cystinuria depends largely on the prevailing interest in the disease Morner s experience in Sweden illustrates this The first case was seen in Sweden in 1870 and the second in 1901 Morner [85] reported 6 seen during the period 1901 to 1920 Sixteen additional cases only 4 without stones were observed in the next 5 years [9] The diagnostic criteria had not changed from the earliest time By 1936 Morner had found a total of 36 cases of cystinuria in the generation of the Swedish population living since 1901 Renander [5] then surveyed independently all hospitals in the country and succeeded in finding only one additional case These studies approach a complete ascertainment of cystinuria in a mean population of 6 million and give a (probably low) incidence of roughly 1 200 000

Between 1929 and 1931 Lewis examined the urine of 10 534 University of Michigan students with the cyanide nitroprusside test and when that was positive by the more specific Sullivan test for cystine [8] The normal excretion of cystine is in the range of 40 to 80 mg per day [88] and approximately the same amount per gram of urinary creatinine [39] It has been estimated that Lewis s cyanide nitroprusside test would be distinctly positive with at least 100 to 125 mg cystine per gram of creatinine [59] Lewis discovered 18 individuals or 1 in 600 who consistently excreted cystine Eleven of these were studied in more detail and all showed an abnormally elevated urinary organic sulfur fraction Four showed repeated cystine crystalluria (4 in 10 000) Earlier estimates of the incidence of cystine crystalluria were less than one fourth this high but they were based on single examinations for a condition known to occur only intermittently None of Lewis s subjects had ever had symptoms suggestive of renal stone or colic and only one gave a family history of such disease A sister of one of the individuals had formed a cystine stone but his parents and five other siblings did not excrete cystine The urine of 22 other individuals in the population studied gave weakly positive tests consistently or occasionally The total incidence of positive tests was 1 in 250 Those giving weakly positive tests had nearly normal sulfur partitions and were considered normal Malleson (cited in [39]) confirmed the high incidence of chemical cystinuria (1 in 250) with the finding of four positive urines from approximately 1 000 students at University College London These urines contained 106 to 161 mg

cystine per gram of creatinine, and paper chromatography demonstrated in all an increased output of lysine as well as cystine

The finding of excess excretion of cystine by 1 in 250 people introduced a new entity, chemical cystinuria An additional criterion besides cystine excretion was obviously necessary to identify the specific disease cystinuria in stoneless persons, and to distinguish it from other conditions which might cause chemical cystinuria The specific aminoaciduria with four amino acids provided the criterion in the absence of stone formation The high frequency of chemical cystinuria found an explanation in the frequency of heterozygotes that can be deduced from studies of the genetics of the disease as determined from the specific aminoaciduria

Dent and Harris undertook a study of cystinuria in terms of the specific aminoaciduria [*89*] The result was simply to redefine what has been called cystinuria as a specific hereditary condition characterized by the presence of the four amino acids in abnormal amounts and to prove that it is distinct from the inherited Fanconi syndrome and from Wilson s disease Studies of the families of three individuals with the latter two diseases disclosed three other individuals with a general aminoaciduria and none with the specific cystine-arginine lysine-ornithine type of aminoaciduria The families of seven classical cystinuric patients, six of whom had stones contained seven more individuals of the same specific type and none with generalized aminoaciduria These findings directly disproved the idea that cystinuria is a stage in the development of the Fanconi syndrome [*76*]

PATTERNS OF INHERITANCE

The familial incidence of cystinuria among siblings was such that Garrod suggested its inheritance as a rare recessive trait Even then however too many instances of direct transmission from parent to child were known and Garrod entertained the possibility that the condition might be dominant An alternative explanation, that the gene is very common instead of rare would also account for the presence of the disease in two successive generations This explanation gained credence when it was known that chemical cystinuria is much more common than the incidence of stone formation

From a consideration of the published pedigrees of cystinuria, complete except for those of Niemann [*6*] Kretschmer [*7*] and Morner [*9, 90*] Dent and Harris concluded that the available data were of little genetic value since only five families had been examined with chemical methods and in none of these was there information about the excretion of other amino acids The data from the earlier studies simply reemphasized the mutually contradictory points made by Garrod the frequency of parental consanguinity and of cases among siblings indicates that cystinuria is a *rare homozygous* recessive disease whereas the presence of affected

individuals in three successive generations of the same family [91] almost certainly indicates that some patients are heterozygotes i e that the condition is sometimes dominant

Analysis of the new family data collected by Dent and Harris did not resolve this problem In two instances the parents of cystinuric patients were normal, so that the condition is not regularly manifested in heterozygotes The occurrence of affected parent child pairs in two of the seven families could be explained on the recessive hypothesis with a postulated high frequency of the gene in the population (one individual in 12 would be a heterozygote if Lewis s incidence of 1 in 600 represented the homozygous form) But if the gene is so common consanguinity would not be important for its expression Yet even in this small series there was one consanguineous marriage It was apparent that the genetics of cystinuria half known for so long on the basis of the qualitative excretion of cystine would yield fully only to a detailed quantitative study

Quantitative studies by Harris and coworkers of the amino acid excretion in cystinuric families provided the answer Measurements in 21 families [39] showed in some of the families the expected clear separation into cystinuric and normal individuals In other families there was a continuous gradation from normal to extremely abnormal cystine excretion in the different individuals It was evident that the disease appeared in two forms, separately inherited

*Recessive Cystinuria*

The first type the more common of the two forms of cystinuria appeared in families where all individuals excreted either normal or highly abnormal amounts of urinary cystine and no individuals had intermediary values There was a sharp segregation for the property of cystine excretion and the family distribution was of the type expected if the cystinuria occurred in individuals homozygous for a rare recessive gene The parents, who were apparently normal were necessarily heterozygotes but the one recessive abnormal gene which they possessed was not strong enough to create any noticeable effect on cystine excretion Thus the defect appeared only among the homozygous offspring Four instances of consanguinity in these families further supported this view This type is called recessive cystinuria

*Incompletely Recessive Cystinuria*

In families of the second type normal intermediate and high values of cystine excretion were all found The family distributions suggested that the individuals with moderately raised values of cystine excretion were heterozygous Those with high values like the affected individuals in the recessive families were homozygous for a rare gene Because the character of the incompletely recessive gene in the second type of

family was strong enough to produce intermediary values in the heterozygotes, it was assumed that the individuals with normal cystine values did not possess the gene for cystinuria Further quantitative studies of the other amino acids excreted made it clear that the earlier contradictory results had found their explanation in two different modes of inheritance of different degrees of the same renal defect

*Effect of Genotype on Renal Tubular Transport*

The variant forms of the disease originate in the inheritance of different degrees of the renal defect This was clear from a study by Harris

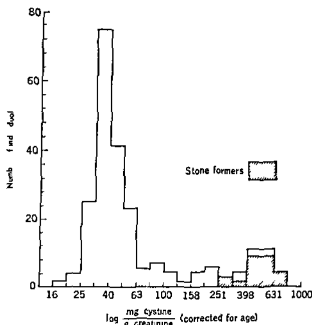



Fig 42 6 Cystine output of cystinuric subjects and their relatives ranged from 15 to 800 mg per gm creatinine Stone formers were clustered among the high cystine excretors (*By permission of H Harris and F L Warren [33]*)

and coworkers of lysine and arginine excretions [*92*] Their patients included 28 stone forming cystinuric persons and 121 of their relatives All degrees of cystinuria from 20 to 800 mg cystine per gram creatinine were found There was stone formation only in individuals who excreted 250 mg cystine per gram creatinine or more This was the level at which a saturating concentration of cystine in urine would be likely to occur (Fig 42 6) The lysine excretion was regularly about twice that of cystine and paralleled the cystine excretion throughout all its gradations Arginine and ornithine were not excreted in abnormal amounts until the cystine and lysine reabsorptions were seriously impaired Only with a

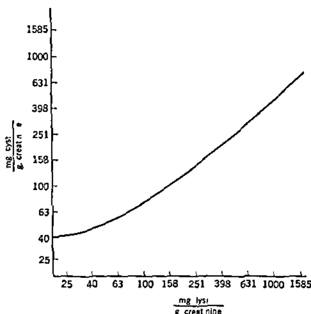
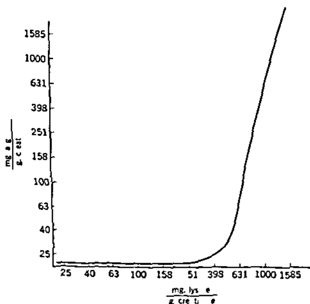



Fig 42-7 Cystine and lysine contents of urine from a series of cystinuric patients and their relatives (*By permiss on of H Harris and E B Robson [94]*)




F g 42-8 A gu e at l ly ine c ntent f urine from a ser es of y t nuric patients an l their latives (*By pe u ion f H Harris a d E B l obsen [ ]*)

cystine excretion of 250 mg per gm creatinine or more did arginine and ornithine also appear in the urine in abnormal amounts The amounts then increased proportionally *with the excretion of higher amounts of* cystine and lysine These relationships, which held in families with both recessive and incompletely recessive cystinuria, are shown graphically in Figs 42 7 and 42 8 Subsequent studies have indicated that the variations in the arginine to lysine ratio are genetically determined in the recessive homozygotes and may indicate still other variants of the disease [*93, 94*]

These genetic studies revealed a hierarchy of affinities of substances for a renal transport system, comparable to the affinities of substrates for an enzyme Arginine and ornithine had high affinities and were preferentially reabsorbed by the tubular mechanism Cystine and lysine *affinities were lower and their reabsorption failed first The genetically* determined degrees of the renal defect, if partial permitted the preferential reabsorption of arginine and ornithine, and if more complete, resulted in the renal loss of all four amino acids including cystine, in sufficient concentration for the latter to precipitate and form stones

*Phenotypes and Genotypes and Incidence of Cystinuria*

The biochemical and genetic data of Harris et al [*39 92*] were finally correlated in a definitive paper Phenotypes and Genotypes in Cystinuria ' [*10*] A total of 29 families each with at least one stone forming cystinuric member was reported Two of these families could not be definitely categorized as to type from the data available but in all families the homozygous individuals were indistinguishable They excreted approximately the same highly abnormal amounts of the four amino acids and nearly all formed cystine stones In 19 of the families *the condition was recessive and no degree of abnormality was* found except the extreme form in the homozygote In 8 families the condition was incompletely recessive and the heterozygote showed moderately increased excretion of cystine and lysine without increased excretion of arginine or ornithine Only in rare instances did the *latter excrete sufficient* cystine to form stones

The simplest explanation of the two variant forms of cystinuria is that there are at least two mutant conditions of one gene One would have a mild and the other a severe effect on the tubular reabsorption of the four amino acids in the heterozygous state Genetic proof of this possibility must await identification of a homozygous cystinuric person one of whose parents is the apparently normal heterozygote of the recessive type and the other a heterozygote of the incompletely recessive type of cystinuria Families evidently of this kind are on record Detailed chemical identification of the type of each individual in such a pedigree *has not* been made

Implicit in the above figures of the relative frequency of the two types is the explanation of the rarity of cystine stone and the commonness of chemical cystinuria The incidence of chemical cystinuria can be accepted at the maximal figure of 1 in 250 and all cases can be attributed to heterozygotes of the rare incompletely recessive form of cystinuria Since the frequency of heterozygotes of a rare disease is approximately $2\sqrt{\text{incidence}}$ of the homozygotes the incidence of incompletely recessive cystinuric homozygotes would be 1 in 250 000 Since recessive cystinuria has been found twice as often as the incompletely recessive type the incidence of cystinuria of both types would then be roughly 1 in 80 000 This is a reasonable figure between the maximum estimates based on crystalluria in selected cases and the ascertainment by Morner of the incidence in Sweden which set the minimal incidence at 1 in 200 000

## DIAGNOSIS

A patient with cystine calculus may be seen at any age and at any stage of the familiar progression of renal or ureteral colic urinary tract obstruction hydronephrosis and secondary bacterial infection Operative interferences and stone recurrences increase the risk of chronic pyelo nephritis progressive renal insufficiency and finally uremia Diagnosis depends upon chemical identification of cystine in the urine or the stone The simple test for cystine in the urine should be performed routinely on all patients suspected of having urinary stones since the stones tend to recur and appropriate treatment may be most beneficial

Cystine stones are about three times as dense to x rays as the poorly visualized urate stones and about equally as dense as the more common calcium magnesium and oxalate stones [o 11] There is nothing char acteristic or diagnostic about the radiographic appearance

### *Microscopic Crystalluria*

Microscopic examination of the urinary sediment for cystine crystals is a most useful diagnostic method The incidence of cystine crystalluria in the population determined by this method is slightly greater than expected for the incidence of the homozygotes Although the present tendency is to use chemical methods whenever possible it is clear that microscopic crystalluria in concentrated (morning) urines is a test suffi ciently sensitive to identify the homozygotes Since stone formation occurs only in (adult) individuals who excrete 0 4 gm or more cystine per day (250 mg per gm creatinine) [92] and no more than this amount dissolves in 1 liter urine at 37°C between pH 5 and 7 it follows that crystals should form when the volume of the 24 hr urine output of potential stone formers is 1 liter or less and the sample is allowed to

stand and cool for several hours before centrifugation If crystals are sought in the relatively concentrated and acid morning urine cystine crystals should be found in the sediment of urines from at least those cystinuric patients who might form stones If a little glacial acetic acid is added (pH 4) and the urine refrigerated overnight the test becomes even more reliable

*Chemical Tests for Cystine*

Although screening tests employing a simple determination of urinary α amino nitrogen promise to be of great value in cystinuria, the simple screening procedure for cystine used by Lewis [*15*] remains the basic clinical test and should be used more widely The cyanide nitroprusside reaction is negative with normal individuals slightly positive with the raised amounts of cystine in urine of heterozygotes of the incompletely recessive disease and strongly positive in urine of the homozygotes Acetone and certain drugs can also give positive reactions

To 5 ml urine add several drops concentrated ammonium hydroxide and 2 ml freshly prepared 5 per cent NaCN Mix and wait 10 min for the reduction of cystine to cysteine Add dropwise some freshly prepared 5 per cent sodium nitroprusside A deep-purple color which fades gradually is a positive result for cystine

The more specific Sullivan reaction can be used for confirmation After reduction of cystine as above add 1 ml freshly prepared 0 5 per cent 1 2-naphthaquinone-4 sodium sulfonate and mix Add 5 ml of 20 per cent anhydrous $Na_2SO$ dissolved in 0 5 N NaOH and mix After 30 min the solution is reddish brown One milliliter of 2 per cent $Na_2S_2O_4$ dissolved in 0 5 *N* NaOH converts the color to a purer red

The method of choice for quantitative cystine studies is polarography [*51*] column chromatographic methods [*81*] are also good

*Identification of Heterozygotes and Homozygotes*

For any purposes other than the purely practical problem of clinical diagnosis chemical identification of the specific aminoaciduria is essential Paper chromatography will distinguish the generalized aminoacidurias of Fanconi s syndrome or Wilson s disease Another common problem is the identification of the heterozygotes of the incompletely recessive form of cystinuria The nature of the problem can be seen from the average values of three amino acids in the urine of normal and cystinuric individuals given in Table 42 1 The family pattern needed to indicate that the two types of cystinuria are allelomorphic should have the excretions of one parent like the controls (heterozygote recessive type) of one parent like the heterozygote of the incompletely recessive type and of one offspring like the homozygote As already mentioned quantitative cystine determinations will help in distinguishing these kinds of

individuals The major distinction is the excretion of grossly abnormal amounts of arginine and ornithine in the homozygotes only they also excrete high amounts of cystine and lysine The presence of abnormal amounts of arginine and ornithine and the very high total α amino nitrogen is therefore characteristic of the homozygote Moderate cystine and lysine excretion without these other characteristics and without *generalized aminoaciduria is found in the heterozygotes of the incom*pletely recessive type

## TREATMENT

Cystinuric patients enjoy excellent health and are long lived except for the complication of urinary stone formation in the homozygous in dividuals The heterozygotes of the incompletely recessive form who account for the vast majority of cystine excretors rarely form stones and need no treatment unless their cystine excretion is over 200 mg per gm creatinine No adverse effect is apparent from the quite considerable daily loss in the urine of the essential amino acids lysine and arginine Subtle effects which might be seen only under stress and during unusual

TABLE 42 1 AVERAGE URINARY AMINO ACIDS

| *Type and No of subjects* | *Cystine* | *Lysine* | *Arginine* | *As α-amino N* |
|---|---|---|---|---|
| Controls (50) | <70 | <100 | < 5 | <16 |
| Heterozygotes (incompletely recessive type) (6) | 1 4 | 101 | < 5 | <28 |
| Homozygotes (both types) (29) | 455 | 1 055 | 39 | 187 |

Expressed in milligrams per gram of creatinine
SOURCE H Harris et al *[10]*

amino acid requirements have not been noticed For example the high lysine requirement for rebuilding hemoglobin after blood loss or the high arginine supply reputedly necessary for growth of spermatozoa might be met with difficulty by the cystinuric patient but observations on these points have yet to be made In one healthy 23 year-old cystinuric person healing of a broken leg was delayed far beyond the usual time but others have undergone major surgical procedures without difficulty The specific aims of treatment should be to prevent initial and recurrent stone formation and when dissolution is preferable to surgical intervention to dissolve stones already formed These aims appear to be attainable

The characteristics which favor the successful treatment and preven tion of cystine lithiasis also make it an ideal model of the study of the general mechanism of stone formation Many factors of an inconstant

stand and cool for several hours before centrifugation If crystals are sought in the relatively concentrated and acid morning urine cystine crystals should be found in the sediment of urines from at least those cystinuric patients who might form stones If a little glacial acetic acid is added (pH 4) and the urine refrigerated overnight the test becomes even more reliable

*Chemical Tests for Cystine*

Although screening tests employing a simple determination of urinary α amino nitrogen promise to be of great value in cystinuria the simple screening procedure for cystine used by Lewis [15] remains the basic clinical test and should be used more widely The cyanide nitroprusside reaction is negative with normal individuals slightly positive with the raised amounts of cystine in urine of heterozygotes of the incompletely recessive disease, and strongly positive in urine of the homozygotes Acetone and certain drugs can also give positive reactions

To 5 ml urine add several drops concentrated ammonium hydroxide and 2 ml freshly prepared 5 per cent NaCN Mix and wait 10 min for the reduction of cystine to cysteine Add dropwise some freshly prepared 5 per cent sodium nitroprusside A deep-purple color which fades gradually is a positive result for cystine

The more specific Sullivan reaction can be used for confirmation After reduction of cystine as above add 1 ml freshly prepared 0 5 per cent 1 2-naphthaquinone-4-sodium sulfonate and mix Add 5 ml of 20 per cent anhydrous $Na_2SO_3$ dissolved in 0 5 N NaOH and mix After 30 min the solution is reddish brown One milliliter of 2 per cent $Na_2S_2O_4$ dissolved in 0 5 N NaOH converts the color to a purer red

The method of choice for quantitative cystine studies is polarography [51] column chromatographic methods [31] are also good

*Identification of Heterozygotes and Homozygotes*

For any purposes other than the purely practical problem of clinical diagnosis chemical identification of the specific aminoaciduria is essential Paper chromatography will distinguish the generalized aminoacidurias of Fanconi s syndrome or Wilson's disease Another common problem is the identification of the heterozygotes of the incompletely recessive form of cystinuria The nature of the problem can be seen from the average values of three amino acids in the urine of normal and cystinuric individuals given in Table 42 1 The family pattern needed to indicate that the two types of cystinuria are allelomorphic should have the excretions of one parent like the controls (heterozygote recessive type) of one parent like the heterozygote of the incompletely recessive type and of one offspring like the homozygote As already mentioned quantitative cystine determinations will help in distinguishing these kinds of

*Solubility of Cystine in Urine*

Major reliance must be placed upon efforts to increase the solubility of cystine in the urine and to increase the volume of urine It was noticed as early as 1890 that cystine is more soluble in urine than in water Blix [97] made a careful study of the increased solubility caused by increased pH and by mineral constituents, diffusible organic materials and colloidal substances The various salts of urine tested individually or in combination increased the amount of cystine which would dissolve by about 50 per cent but Blix attributed the major effect to colloidal substances remaining in dialyzed urine which dissolved twice as much cystine as did water

An elegant study of the solubilizing effect of NaCl and of glycine on cystine was published by Cohn McMeekin and Blanchard [74] The profound solvent action of either of these substances nearly doubled the solubility of cystine but effects were not additive Dent and Senior [16] found no increased solubility by concentrations of urea up to 10 gm per 100 ml This was suggested by the high solubility of cystine in the urea rich urine of the Kenya genet [69] NaCl other amino acids and hippuric acid also failed to increase the solubility of cystine in urine The only prospects of producing a clinically significant increase in cystine solubility in urine appear to depend upon pH and upon possible colloidal solubilizers [70] or complexes of cystine There is no real evidence for the existence of a more soluble complex form of cystine but in view of the striking solubilities reported in genet urine the subject warrants further study

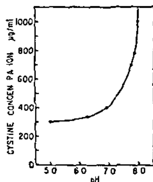



Fig 42-9 The solubility of cystine in urine at 37 C with different pH s (*Redrawn from C E Dent and B Senior [16]*) Cystine is about half as soluble in water

There is an important effect of pH on the solubility of cystine in urine (Fig 42 9) Unfortunately little of it is in the physiologic range From pH 5 to 7 the increase in solubility is only from 0 31 gm to 0 40 gm per liter [16] To obtain a significant effect the urine pH must be held near the maximum of alkalinity and must certainly be above pH 7 5 This is greater alkalinity than can be produced by a high vegetable and fruit diet alone The clinical problem is comparable to that of preventing precipitation in the kidney of the early sulfonamide compounds Sufficient clinical experience with intensive alkali therapy is available to warrant its use at least under hospital supervision even for dissolving stones Carbonic anhydrase inhibitors like Diamox greatly simplify the production of an alkaline urine for limited periods Intensive alkali therapy

nature, such as diet infection and urinary tract abnormalities, may be concerned with the seeding, propagation, and placement of stones in the urinary tract At the present time however, stone formation is best considered as due to simple precipitation from supersaturated solutions It is probable that this mechanism is the one of primary importance in cystinuria even if not in other calculous diseases, since there is a close correlation between the amount of cystine excreted and the tendency to form stones Most individuals who excrete sufficient cystine ultimately develop stones Certainly the concentration of cystine in the urine is the single overwhelmingly important factor in the genesis of these stones Crystallization can be minimized by reducing the cystine concentration either by reducing the amount or by increasing the urine volume, or by increasing the solubility of cystine

*Quantity of Cystine in Urine*

Only a small degree of control over the amount of cystine excreted can be expected A variety of ingenious means have been tried without success to draw cystine off into metabolic bypaths [6, 96] but the only effective way of diminishing the amount of cystine excreted is by dietary protein restriction a method advocated for as long as cystine has been identified as a constituent of protein The fact that more cystine is excreted on high protein diets was not fully accepted despite positive proof [25] until the experiments of Brand et al [41] This is because the effect of protein is small and the amount of cystine in urine merely a reflection of its blood concentration The blood level is maintained from tissue as well as dietary sources, and surpluses can be metabolized Only the methionine in the protein not the cystine significantly raises the blood level of cystine Severe protein restriction will only cause weight loss and negative nitrogen balance which like fever will increase the breakdown of body protein and the excretion of cystine Excess protein should be avoided but enough must be given to replace the essential amino acids continually lost in the urine in this disease

It is possible that high protein diets were the usual fare of two unusual patients who regularly excreted about 3 gm cystine per day at least twice the amount usually found in cystinuria [96] Fluid and alkali therapy were inadequate to prevent stone formation in these patients and dietary measures limiting methionine intake were successfully used to decrease cystine output to the point where other therapies controlled the crystallization The diet used was only moderately low in protein, but the proteins were substantially of vegetable origin since most of the dietary methionine is obtained from animal proteins This diet more than halved the cystine output in these patients and was continued without harm for as long as 18 months

normally secreted during the night constitutes the greatest hindrance to this plan

The regular occurrence of crystalluria in the concentrated morning urines of cystinuric patients was recognized early Indeed it was believed that the formation of stones takes place only at night [7, 101, 102] and Dent and Senior made this the basis of their treatment regimen [16] They collected separately the day and night urines of a cystinuric patient and measured the cystine excreted and the urine flow Averaged results

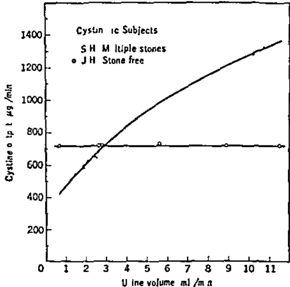



Fig 42-10 Cystine output per minute at various rates of urine flow in stone-free cystinuric (O) and cystinuric with stones (●) Increased output with higher flow in latter patient indicates dissolution of stone by diuresis (*By permission of C F Dent and B Senior [16]*)

from their study appear in Table 42 2 There was a constant rate of cystine excretion throughout the 24 hr During the day the urine flow was adequate to keep the cystine in solution but at night the decreased urine flow resulted in supersaturation and crystallization even at 37 C Additional experiments are shown in Fig 42 11

Other factors may also be necessary for actual precipitation and accretion of stones The occurrence of first stones in cystinuric persons at almost any age suggests that all the necessary conditions for stone formation are met infrequently in the day to-day life of any one individual It is probable that stones form and grow at night Perhaps the dilute day urine washes away the nightly deposition When this undependable equilibrium fails the cystine stones can grow very rapidly indeed Not rarely there are recurrences of cystine stones before the patient is dis-

must not be maintained too long however, because of the danger of producing phosphate stones

Alkali therapy was used as early as 1880 by Cantani [98] He used potassium bicarbonate or citrate in his single patient Klemperer and Jacoby used the alkali treatment successfully to dissolve a formed stone in 1914 [99] Others have reported similar successes with sodium citrate or bicarbonate and in some instances have presented radiologic documentation of the dissolution of the stones in as short a period as 1 month Moderate doses of sodium bicarbonate and water diuresis for 6 weeks enabled one patient to pass 284 stones weighing 10 4 gm in the aggregate [16] The fact that alkali administration increases the solubility of cystine in vivo is further attested by one of the curious misconceptions perpetuated in the medical literature When cystine excretion was recognized primarily by the presence of crystals in urine alkali therapy was seen to dissolve the crystals and accordingly was given the credit for stopping the cystinuria! In 1929 [73] and again as late as 1935 [71] it was found necessary to prove that the disappearance of the crystals of cystine during treatment with sodium bicarbonate or citrate does not signify a reduction in cystine output The limited surface of a stone available for solvent action gives such an unfavorable prospect that the few stones successfully dissolved away are the more remarkable The prevention of first or recurrent stones would be better achieved by alkali and diuresis than stone removal As discussed below alkalinization, like diuresis must be maintained to be effective and this requires medication during the night

Dent and Senior were of the opinion that alkali therapy was not necessary if adequate diuresis was maintained [16] The dissolution of cystine stones in vivo by diuresis alone had been reported by Weinberg and Tabenkin [100] as well as by Dent and Senior [16] In the latter experiments the rates of cystine excretion were compared in two patients one with renal cystine stones and one stone free (Fig 42 10) As the rates of urine flow were increased from 1 to 11 ml per min by water diuresis, the amount of cystine excreted per minute by the stone free patient remained constant although the concentration fell considerably The patient with cystine stones excreted more cystine per minute as the rate of urine flow increased The concentration of cystine in the latter urine tended to be kept nearly constant by the stone cystine which dissolved

Diuresis in the past has usually been combined with alkali therapy The older data available do not permit crediting one or the other with successes achieved but the experiments just cited and the quantitative considerations of cystine excretion and solubility indicate that diuresis is the correct basis of therapy Any regimen should be so designed that it can be adhered to for years without failure and that it will maintain the desired conditions in the renal collecting tubules and in the bladder throughout the 24 hr The relatively concentrated and acidic urine

below 500 μg per ml have been maintained in this way throughout the 24-hr period Under these conditions there are no supersaturated urines and no stone deposition Any stones already present slowly dissolve

## SUMMARY

1 Cystinuria is an inherited condition of man characterized by excessive appearance in the urine of cystine lysine arginine and ornithine No other amino acids are found in excessive amounts The condition is without deleterious effect on the subject except that cystine tends to precipitate and form stones in the urinary passages with pathologic consequences

2 Cystine stones comprise approximately 1 per cent of all stones of the urinary passage Cystine crystalluria is formed in approximately 0 01 per cent of pathologic urine specimens Chemical cystinuria is found in approximately 1 out of every 250 individuals

3 The nature of the renal tubular transport of cystine is entirely unknown The clearance of cystine in normal subjects is approximately 3 ml per min, whereas it may approach the glomerular filtration rate in patients with cystinuria A favored hypothesis to account for the disease is that a receptor binding substance for the four dibasic amino acids is missing in the homozygous cystinuric person and is present in reduced amounts in the heterozygote

4 There appear to be two forms of cystinuria In one the inheritance pattern is recessive and heterozygotes show no noteworthy excess of cystine in the urine In the other the inheritance is incompletely recessive and heterozygotes show intermediate degrees of cystinuria and may rarely form stones Possibly the two variants of the disease derive from variant abnormalities of allelic genes

5 Cystinuria would be an entirely benign disease were it not for the low solubility of cystine especially in neutral or acid solution Preventive treatment in the homozygote is directed toward avoiding excess methionine intake maintaining an alkaline urine by frequent use of alkali and an alkaline ash diet and more importantly by maintaining at all times a rapid flow of urine It is particularly important to maintain a large volume of urine during the sleeping hours when normally the urine becomes more concentrated and cystine tends to precipitate Much can be done with this same regimen to dissolve cystine stones which have already formed

6 Cystine excretion occurs in the Fanconi syndrome and in Wilson s disease as part of the general aminoaciduria of those diseases It is seen as a species wide isolated aminoaciduria in the blotched genet a wild cat of Kenya It is seen occasionally in several varieties of the dog but the exact nature of canine cystinuria remains to be ascertained Recently cystine urinary stones have been encountered in the mink

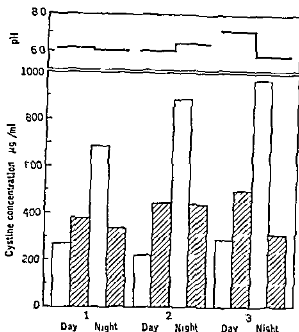



Fig 42-11 The nighttime excretion of urine super aturated with cystine pH and cystine concentrations of day and night urines of a cystinuric person over three 24-hr periods (clear columns) are shown Amount of cystine which could dissolve in true solution in each urine is shown by hatched areas (*By permission of C E Dent and B Senior [16]*)

charged from the hospital following a stone removal Even high rates of diuresis of over 3 liters per 24 hr produced by daytime water dosage, do not avoid the relatively concentrated urine of the early morning hours

TABLE 42 2 DAY AND NIGHT VARIATION IN URINARY CYSTINE CONCENTRATION IN CYSTINURIA

| *Period* | *Rate of excretion* | | *Cystine concentration μg/ml* |
|---|---|---|---|
| | *Cystine μg/min* | *Urine ml/min* | |
| 6 A M 10 P M | 538 | 2 1 | 256 (undersaturated) |
| 10 P M -6 A M | 492 | 0 7 | 703 (super aturated) |

SOURCE C E Dent and B Senior [16]

Dent and Senior recommended a regimen of diuresis which avoided the supersaturated urines of early morning In addition to a high daytime water intake (500 ml every 4 hr) patients were instructed to drink two glasses of water at bedtime and two more at 2 A M Periodic tests of the cystine concentration of morning urines were used to check the success of the procedure Urine flows of 2 ml per min with cystine concentrations

30 Eberlein W R Aminoaciduria in childhood cystinuria and cystinosis Am J M Sc 225 677 1953
31 Morner K A H Cystin ein Spaltungsprodukt der Harn ul tanz Ztschr physiol Chem 28 595 1899
32 Friedman E Der Kreislauf des Schwefels in der organi ch en Natur Engel n Physiol 1 15 1902
33 Tennant C E Cystin calculi a complex surgical problem J A M A 80 305 1923
34 *Gortner R A and Hoffman W F Sulfur in proteins III Derivatives of l* and i-cystine J Biol Chem 72 433 19 
35 Thiele F H Concerning cystinuria and diamines J Physiol 36 68 1907
36 Garrod A E Anomalies of urinary excretion in O lers *Modern Medicine* vol 6 p 71 Lea & Febiger Philadelphia 1909
37 Pfeiffer E Eine Cystinfamilie Centralbl Krankh Harn u Sex Org 8 1 3 1897
38 Cohn J Über familiare Cystinurie Berlin klin Wchnschr 36 503 1899
39 Harris H and Warren F L Quantitative studies on the urinary cystine in patients with cystine stones and their relatives Ann Eugenics 18 1 5 1953
40 Brand E Cahill G F and Harris M M Cystinuria II The metabolism of cystine cysteine methionine and glutathione J Biol Chem 109 69 1935
41 Brand E Block R J Kassell B and Cahill G F Cystinuria V The metabolism of casein and lactalbumin J Biol Chem 119 669 1937
42 Brand E and Cahill G F Further studies on metabolism of sulfur compounds in cystinuria Proc Soc Exper Biol & Med 31 1247 1934
43 Brand E Block R J Kassell B and Cahill G F Carboxymethylcysteine metabolism its implications on therapy in cystinuria and on the methionine cystine relationship Proc Soc Exper Biol & Med 35 501 1936
44 du Vigneaud V Kilmer G W Rachele J R and Cohn M On the mechanism of the conversion in vivo of methionine to cystine J Biol Chem 155 645 1944
45 Rachele J R Reed L J Kidwai A R Ferger M F and du Vigneaud V Conversion of cystathionine labeled with S to cystine in vivo J Biol Chem 185 817 1950
46 Horowitz N H Methionine synthesis in *Neurospora* The isolation of cystathionine J Biol Chem 171 255 1947
47 Blum L Über das Schicksal des Cystins im Tierkorper Beitr chem Physiol u Path 5 1 1904
48 Wolf C G L and Shaffer P A Protein metabolism in cystinuria *J Biol* Chem 4 439 1908
49 Brown B H and Lewis H B Cystine in normal and cystinuric human blood Proc Soc Exper Biol & Med 36 487 1937
0 Patch F S Cystinuria and cystine lithiasis Canad M A J 31 250 1934
51 Dent C E Senior B and Walshe J M The pathogenesis of cystinuria II Polarographic studies of the metabolism of sulfur-containing amino acids J Clin Invest 33 1216 1954
52 Ellinger A Bildung von Putrescin aus Ornithin Ber deuts h chem Gesellsch 31 3183 1898
53 von Udránszky L and Baumann E Ueber das Vorkommen von Diamin n sogenannten Ptomainen bei Cystinurie Ztschr physiol Chem 13 56 1889
54 von Udránszky L and Baumann E Beitrage zur Kenntni der Cystinurie Ztschr physiol Chem 15 77 1891
55 Garcia S A Über Ptomaine Ztschr physiol Chem 17 577 1893
55a Ackermann D and Kutscher F Ueber das Vorkommen von Lysin im Harn bei Cystinurie Ztschr Biol 57 355 1911
56 Hoppe Seyler F A Lysinausscheidung im Harn bei Cystinurie Ztschr physiol Chem 214 267 1933
57 Lassaigne J L Observation sur l existence de l oxide cystique dans un calcul vesical du chien et essai analytique sur la composition elémentaire de cette substance p rticulière Ann chim et phys 2d se es 23 328 1823

# BIBLIOGRAPHY


1 Garrod A E Inborn errors of metabolism Lancet 2 1908 Lecture I p 1 Lecture II p 73 Lecture III p 142 Lecture IV p 214
2 Loewy A and Neuberg C Über Cystinurie Ztschr physiol Chem 43 338, 1904
3 Lewis H B Brown B H and White F R The metabolism of sulfur XXIII The influence of the ingestion of cystine cysteine and methionine on the excretion of cystine in cystinuria J Biol Chem 114 171 1936
4 Wollaston W H On cystic oxide A new species of urinary calculus Tr Roy Soc London 100 223 1810
5 Renander A The roentgen density of the cystine calculus Acta radiol Suppl 41 Stockholm 1941
6 Niemann A Beitrag zur Lehre von Cystinurie beim Menschen Deutsches Arch klin Med 18 232 1876
7 Kretschmer H L Cystinuria and cystine stones with report of a new family of cystinurics Urol & Cutan Rev 20 1 1916
8 Lewis H B The occurrence of cystinuria in healthy young men and women Ann Int Med 6 183 1932
9 Morner C T On present knowledge of cystinuria Upsala lakaref forh 31 171 1925–1926
10 Harris H Mittwoch U Robson E B and Warren F L Phenotypes and genotypes in cystinuria Ann Human Genet 20 57 1955
11 Morrison H R A roentgenological study of cystine urinary calculi Am J Roentgenol 44 537 1940
12 Meyer L Stoffwechseluntersuchungen bei Cystinurie Deutsches Arch klin Med 172 207 1931
13 Thompson H Large cystic oxide calculus successfully removed by lithotrity in a patient aged 81 years Tr Path Soc London 21 272 1870
14. Marowsky Ein Fall von Cystin im Harn Deutsches Arch klin Med 4 449 1868
15 Lewis H B Cystinuria A review of some recent investigations Yale J Biol 4 437 1932
16 Dent C E and Senior B Studies on the treatment of cystinuria Brit J Urol 27 317 1955
17 Ebstein W Die Natur und Behandlung der Harnstein J F Bermann Wiesbaden 1884
18 Simon C E Cystinuria and its relation to diaminuria Am J M Sc 119 39 1900
19 Wasserthal Beitrag zur Kasuistik und Atiologie der Cystinurie Centralbl Krankh Harn u Sex Org 15 121 1904
20 von Hoffmann K Die Cystinurie Zentralbl Grenzgeb Med u Chir 9 321 1907
21 Link R Beitrag zur Kenntnis der Cystinurie und der Cystinsteine Inaugural Dissertation Sturm & Koppe Leipzig 1912
22 Sum Schick L Zur Cystinuriefrage Ztschr Urol 23 985 1929
23 Southam F A Two cases of cystine calculi Brit M J 1 489 1907
24 Looney J M Berglund H and Graves R C A study of several cases of cystinuria J Biol Chem 57 515 1923
25 Alsberg C and Folin O Protein metabolism in cystinuria Am J Physiol 14 54 1905
26 Harrison R Case of lithotomy where a large cystic oxide calculus was removed Brit M J 2 10 1879
27 Andersen I Cystinuri og cystinsten Nord Med 19 397 1941
28 Abderhalden E Familiare Cystindiathese Ztschr physiol Chem 38 557 1903
29 Lignac Leiden G O E Cystinbefunde bei einer bestimmten Kinderkrankheit Verhandl deut ch path Gesellsch 21 303 1926

84 Nakano H *Atlas der Harnsteine* F Deutcke Leipzig 1925
85 Morner C T Cystin calculi Upsala lakaref forh 26 65 1920 Abstract in J A M A 76 560 1921
86 Garrod A E *Inborn Errors of Metabolism*, 2d ed Froude Hodder and Stoughton London 1923
87 Sondern F E Cystinuria *Arch Diagnosis* 4 267 1911
88 Reed C The polarographic estimation of cystine in urine J Biol Chem 142 61 1942
89 Dent C E and Harris H Genetics of cystinuria Ann Eugenics 16 60 1951
90 Morner C T Utredning av vad hitintills kaut är om Cystinuri inom vårt land Upsala lakaref forh 37 205 1931
91 Andrews J C and Brooks R E Study of cystinuric family J Urol 47 171 1942
92 Harris H Mittwoch U Robson E B and Warren F L Pattern of amino acid excretion in cystinuria Ann Human Genet 19 196 1955
93 Harris H and Robson E B Variation in homozygous cystinuria Acta Genet 5 381 1955
94 Harris H and Robson E B Cystinuria Am J Med 22 774 1957
95 Harper H A Hutchin M E and Kimmel J Concentrations of 19 amino acids in plasma and urine *Proc Soc Exper Biol & Med* 80 768 1952
96 Smith D R Kolb F O and Harper H A Management of cystinuria and cystine-stone disease J Urol 81 61 1959
97 Blix G Über die Laslichkeitsverhaltnisse on Cystin im Harn Ztschr physiol Chem 178 109 1928
98 Cantani A Specielle Pathologie and Therapie der Stoffwechselkrankheiten Band II Oxalurie Gicht und Steinkrankheiten Denicke Berlin 1880
99 Klemperer G and Jacoby M *Zur Behandlung der Cystinurie Die Therapie der Gegenwart* Bd 55 101 Berlin 1914
100 Weinberg S R and Tabenkin P A Observations on therapy of cystine calculus disease Arch Int Med 90 850 1952
101 Bartels Ein Fall von Cystinurie Virchows Arch path Anat 26 419 1863
102 Toel F Beobachtungen uber Cystinbildung Ann Chem & Pharmacol 96 247 1855
103 Hele T S Metabolism in cystinuria *J Physiol* 39 52 1909 1910

58 Gross S W Case of cystine calculus in a dog North Am Medico-Chirurgical Rev 5 311 1861
59 Lentz W J A rare form of urolithiasis associated with clonic spasms of muscles (chorea) of the posterior limbs of the dog J Am Vet M A 59 365 1921
60 Morris M L Green D F Dinkel J H and Brand E Canine cystinuria North Am Vet 16 16 1935
61 Brand E and Cahill G F Canine cystinuria III J Biol Chem 114, 25 1936
62 Brand E Cahill G F and Kassell B Canine cystinuria V Family history of two cystinuric Irish terriers and cystine determinations in dog urine J Biol Chem 133 431 1940
63 Hess W C and Sullivan M X Canine cystinuria The effect of feeding cystine cysteine and methionine at different dietary protein levels J Biol Chem 143 545 1942
64 Green D F Morris M L Cahill G F and Brand E Canine cystinuria II Analysis of cystine calculi and sulfur distribution in the urine J Biol Chem 114 31 1936
65 Beyer K H Wright L D Skeggs H R Russo H F and Shaner G A Renal clearance of essential amino acids their competition for reabsorption by the renal tubules Am J Physiol 151 202 1947
66 Krabbe A Urolithiasis in dogs and cats Vet Rec 61 751 1949
67 White E G Urinary calculi in the dog with especial reference to cystine stones J Comp Path 54 16 1944
68 Crane C W and Turner A W Amino acid patterns of urine and blood plasma in a cystinuric Labrador dog Nature London 177 237 1956
69 Datta S P and Harris H Urinary amino acid patterns of some mammals Ann Eugenics 18 107 1953
70 Brand E Harris M M and Biloon S Cystinuria Excretion of a cystine complex which decomposes in the urine with the liberation of free cystine J Biol Chem 86 315 1930
71 Andrews J C and Randall A Sulfur metabolism in cystinuria J Clin Invest 14 517 1935
72 Doolan P D Harper H A Hutchin M E and Alpen E L Renal clearance of lysine in cystinuria pathogenesis and management of this abnormality Am J Med 23 416 1957
73 Robson W Protein metabolism in cystinuria Biochem J 23 138 1929
74 Cohn E J McMeekin T L and Blanchard M H The solubility of cystine in the presence of ions and another dipolar ion J Gen Physiol 21 651 1937–1938
75 Oldfield J E Allen P H and Adair J Identification of cystine calculi in mink Proc Soc Exper Biol & Med 91 560 1956
76 Yeh H L Frankl W Dunn M S Parker P Hughes B and Gyorgy P The urinary excretion of amino acids by a cystinuric subject Am J M Sc 214 507 1947
77 Stein W H Excretion of amino acids in cystinuria Proc Soc Exper Biol & Med 78 705 1951
78 Dent C E and Rose G A Amino acid metabolism in cystinuria Quart J Med 20 205 1951
79 Fowler D Harris H and Warren F L Plasma-cystine levels in cystinuria Lancet 1 544 1952
80 Dent C E Heathcote J G and Joron G E The pathogenesis of cystinuria I Chromatographic and microbiological studies of the metabolism of sulfur containing amino acids J Clin Invest 33 1210 1954
81 Stein W H and Moore S The free amino acids of human blood plasma J Biol Chem 211 915 1954
82 Robson E B and Rose G A The effect of intravenous lysine on the renal clearance of cystine arginine and ornithine in normal subjects in patients with cystinuria and Fanconi syndrome and in their relatives Clin Sc 16 75 1957
83 Becker F F and Green H Incorporation of cystine and lysine by normal and cystinuric leukocytes Proc Soc Exper Biol & Med 99 694 1958

pellagra like rash was most severe The excretion pattern of amino acids in the urine was identical with that of her brother, E H

It was clear then that these two siblings were affected by the same disease An inherited condition seemed probable when it was learned that the parents were first cousins

*The Hartnup Family*

Neither parent and none of their six other children gave a clinical history to sugge t that they were similarly affected although one girl

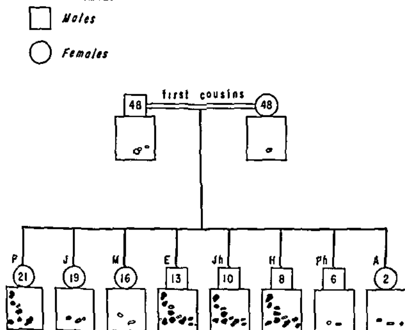



Fig 43 1 The genealogy of Hartnup disease as illu trated in the Hartnup family The parents were first cousins The age of the subject in 1953 is ndicated by the numbers in the squares are illustrated the chromatographic patte ns of urine samples in two-dimensional chromatograms sta ned for am no acids

(M H ) was mentally retarded However two younger siblings of E H Jh H and H H also had gross aminoaciduria with the characteristic chromatographic pattern No abnormality was detected in the urine of the other four siblings or in either parent In the affected children the amino acid excretion has persisted unchanged in pattern and amount up to the present The skin and neurologic disturbances have gradually lessened in P H and E H but have made a fleeting appearance in the

# Chapter 43

## Hartnup Disease*

---

*John B Jepson and Mary Jane Spiro*

Hartnup disease is a familial condition characterized by aminoaciduria and other abnormalities apparently associated with impaired amino acid transport The publication which first fully reported the existence of this disease [1] bore the title "Hereditary Pellagra like Skin Rash with Temporary Cerebellar Ataxia Constant Renal Amino Aciduria and Other Bizarre Biochemical Features As a description of the main aspects of the disease this title cannot be bettered The family in which the condition was first found consented to the use of their surname *Hartnup*, as an appropriate appellation The original publication [1] referred only obliquely to the name Hartnup and used the term *H disease* occasional reference had earlier been made to *Hart s syndrome*

### HISTORY

In 1951 a boy aged 12 E Hartnup was admitted to the Middlesex Hospital, London Eng with mild cerebellar ataxia and a red scaly rash on the exposed areas of his body His mother avowed that he had pellagra for her eldest daughter (P H ) with identical symptoms had been treated at the hospital in 1937 for that disease Although the rash in F H was quite consistent with pellagra, other findings were not and a diagnosis of pellagra as a *dietary* deficiency disease was untenable

Apart from variable cerebellar signs and retarded mental development the only abnormality detected at that time was in the urinary excretion of free amino acids Paper chromatography of the urine disclosed an excretion pattern of amino acids quite unlike that seen in any other disease

At about the same time P H now aged 19 had a recurrence of ataxia without a rash similar to that which she had had in childhood when the

* Dr Spiro s work was supported in part by The Medical Foundation Inc

TABLE 43.1 THE KNOWN CASES OF HARTNUP DISEASE

| Investigators | Relationship of parents | No. of children | Designation of patient | Sex | Year of birth | Position in family | Clinical (age when symptom first noticed) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Ataxia | Rash | Mental status |
| [1] | First cousin (Hartnup family) | 8 | I H | F | 193[illegible] | 1st | Yes (5) | Yes (3) | Retarded |
| | | | E H | M | 1939 | 4th | Yes (1[illegible]) | Yes ([illegible]) | Retarded |
| | | | Jh H | M | 1943 | 5th | Yes (11) | Yes (8) | Fair |
| | | | H H | M | 1945 | 6th | No | No | High |
| [1 2] | Not reported | 1 | Not reported (initials T S ) | F | 1949 | Only | No | Yes (4) | Normal |
| [3–6] | Unrelated | 4 | M H | M | 194[illegible] | 4th | Slight | Yes (10) | Psychosis (now normal) |
| [[illegible]] | Unrelated | 2 | Angela [illegible] | F | 194[illegible] | 2d | Yes (5) | Yes ([illegible]) | Normal |
| [[illegible]] | Second cousins | 9 | D | F | 1948 | [illegible]th | Yes | Yes (3) | Low |
| | | | Sister of D | F | 1954 | 9th | Yes | Yes (3) | Normal |
| [7] | Related but not specified | 3 | C | F | 1947 | 1st | Yes (5) | Yes (6) | Below normal |
| [[illegible]] | Unrelated | 3 | J | F | 1931 | 1st | Yes (18) | Yes (9) | High |
| | | | E | F | 193[illegible] | 2d | Yes (17) | Yes (8) | Normal |
| [8] | Unrelated | [illegible] | Cundren B | F | 194[illegible] | 1st | No | Yes (7) | Normal |

younger boys The mental defect in M H does not seem related to the main abnormality

The pedigree of the Hartnup family (as of 1953), with diagrammatic representation of the chromatographic findings, is given in Fig 43 1 No other relatives of the Hartnup parents show the abnormality

*Other Cases*

After publication of the definitive report on Hartnup disease in 1956 [1], two cases of 'pellagra" in English children which had been earlier reported [2, 3] were reexamined In the first case, described by Hickish [2], the dietary history was not entirely convincing The second [3 4] was a child with acute psychosis and large quantities of indican in the urine Chromatography of the urines [1] indicated that these two patients were not true dietary pellagrins but had Hartnup disease Seven other cases of Hartnup disease since discovered bring the total to 13 (Table 43 1)

## CLINICAL ASPECTS

The clinical manifestations of Hartnup disease are both intermittent and variable Although the biochemical lesion as represented by amino aciduria is always present clinically recognizable attacks' may occur only rarely and in widely different forms

*Skin Lesions*

In the original Hartnup cases and in most of those found subsequently, the primary cause of referral to hospital has been a red scaly rash, sometimes dry sometimes raw and blistered The rash appears intermittently usually in summertime with a distribution on exposed parts of the face neck hands and legs suggesting photosensitivity [1 6] Hartnup patients learn to avoid exposure to direct light but photosensitivity has not been convincingly demonstrated by experiment The dermatitis closely resembles that seen in dietary pellagra in appearance and distribution

*Neuropsychiatric Manifestations*

Several of the patients with Hartnup disease have developed a severe but fully reversible cerebellar ataxia These neurologic crises seem to be precipitated at those times when the skin rash is most severe but occasionally they may follow infectious disease without rash [1 6 7] During attacks the patient has an unsteady gait and walks with a wide base Arm movements are jerky and there is an intention tremor Nystagmus and double vision are present No sensory abnormalities are found The ataxia develops suddenly and rapidly reaches a peak The manifestations may vary from day to day Gradual improvement follows over a period of weeks and complete recovery is the rule

younger boys The mental defect in M H does not seem related to the main abnormality

The pedigree of the Hartnup family (as of 1953) with diagrammatic representation of the chromatographic findings is given in Fig 43 1 No other relatives of the Hartnup parents show the abnormality

*Other Cases*

After publication of the definitive report on Hartnup disease in 1956 [1] two cases of "pellagra" in English children which had been earlier reported [2 3] were reexamined In the first case, described by Hickish [2] the dietary history was not entirely convincing The second [3 4] was a child with acute psychosis and large quantities of indican in the urine Chromatography of the urines [1] indicated that these two patients were not true dietary pellagrins but had Hartnup disease Seven other cases of Hartnup disease since discovered bring the total to 13 (Table 43 1)

## CLINICAL ASPECTS

The clinical manifestations of Hartnup disease are both intermittent and variable Although the biochemical lesion as represented by amino aciduria is always present clinically recognizable 'attacks' may occur only rarely and in widely different forms

*Skin Lesions*

In the original Hartnup cases and in most of those found subsequently the primary cause of referral to hospital has been a red scaly rash sometimes dry sometimes raw and blistered The rash appears intermittently usually in summertime with a distribution on exposed parts of the face neck hands and legs suggesting photosensitivity [1 6] Hartnup patients learn to avoid exposure to direct light but photosensitivity has not been convincingly demonstrated by experiment The dermatitis closely resembles that seen in dietary pellagra in appearance and distribution

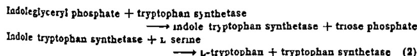
*Neuropsychiatric Manifestations*

Several of the patients with Hartnup disease have developed a severe but fully reversible cerebellar ataxia These neurologic crises seem to be precipitated at those times when the skin rash is most severe but occasionally they may follow infectious disease without rash [1 6 7] During attacks the patient has an unsteady gait and walks with a wide base Arm movements are jerky and there is an intention tremor Nystagmus and double vision are present No sensory abnormalities are found The ataxia develops suddenly and rapidly reaches a peak The manifestations may vary from day to day Gradual improvement follows over a period of weeks and complete recovery is the rule

microorganisms by utilizing various mutant strains lacking one or another of the enzymes in the sequence of tryptophan synthesizing enzymes The synthesis of the indole ring of the tryptophan molecule appears to involve the condensation of anthranilic acid with a phosphorylated sugar derivative as shown in Eq (1)


**Anthranilic acid + phosphoribosylpyrophosphate ⟶ indoleglyceryl phosphate (1)**

This pathway occurs in *Escherichia coli* [9] *Aerobacter aerogenes* [10] and *Neurospora crassa* [11] Some strains of *Saccharomyces* appear to utilize a phosphorylated hexose derivative instead of the pentose phosphate [12] The conversion of indole and L-serine to L-tryptophan by the enzyme tryptophan synthetase a pyridoxal phosphate requiring enzyme has been well established [13] Recently however Yanofsky [11] has proposed the direct utilization of the indoleglyceryl phosphate formed in Reaction (1) without prior splitting to indole and triose phosphate The reaction as shown in Eq (2) would involve an intermediate step with the indole group attached to the enzyme


**Indoleglyceryl phosphate + tryptophan synthetase**
**⟶ indole tryptophan synthetase + triose phosphate**
**Indole tryptophan synthetase + L serine**
**⟶ L-tryptophan + tryptophan synthetase (2)**

*Degradation*

The degradation of tryptophan is of particular interest because of its ultimate conversion to the physiologically important compounds nicotinic acid and serotonin Figure 43 2 shows the many pathways by which tryptophan may be catabolized

The over all metabolism of tryptophan in the intact animal has been assessed by the administration of $C^{14}$ labeled tryptophan to the rat [14 15] Because of the rapid conversion of both uniformly labeled and ring labeled tryptophan to $C^{14}O_2$ and to various Krebs cycle-derived amino acids it has been proposed that the most important catabolic reaction of tryptophan may be the cleavage of the benzene ring at the level of 3 hydroxyanthranilic acid with the formation of acetate and other aliphatic intermediates which are finally converted to $CO_2$ Further support for this view has been provided Gholson and Henderson using an acetate trapping technique [16] found the conversion of the 7 α carbon of tryptophan to the carboxyl carbon of acetate In addition Hankes and Henderson [17] found that carboxyl labeled 3 hydroxy anthranilic acid was converted to $C^{14}O_2$ much more rapidly than could be accounted for by decarboxylation of this compound or its products

Formylkynurenine Tryptophan is first converted to formylkynurenine by the enzyme tryptophan pyrrolase This reaction has been studied in liver by Knox and Mehler [18 19] and by Hayaishi and Stanier in

Some patients have never shown severe ataxia, but have experienced a period of "collapsing" or fainting attacks without warning [9] In the case reported by Bickel [8], cerebral involvement was limited to stubborn headache

Psychiatric features have ranged from mild emotional instability to complete delirium, a range similar to that of the psychiatric disturbances of classical pellagra They were the cause for referral to the hospital in only two cases—bizarre delusions and vivid hallucinations in one case [3], and depersonalization in the other (Patient E) [9] The intelligence quotients of the four affected children of the original Hartnup family ranged from 101 in the youngest to 61 in the eldest Baron et al [1] suggested that the biochemical lesion causes progressive mental deterioration, but cases discovered more recently do not lend support to this idea for several of the older patients have average or higher than average intelligence and ability [9 8] Furthermore the younger Hartnup children have shown no further signs of deterioration, and the older ones have if anything improved

*Precipitating Factors*

Attacks have been precipitated by fever [1] sulfonamide therapy [6] and psychologic stress [9] In every case there was a history of inadequate or irregular diet (e g one patient had lived for months largely on corn flakes [6]) It may be that clinical signs seldom appear unless under the provocation of poor nutrition A variation with age or stress in requirement for some nutrient which is in limited supply because of a biochemical abnormality might explain the clinical deterioration with growth and improvement in adult life This limiting nutrient may be nicotinic acid accordingly a discussion of tryptophan metabolism and the metabolic origins of nicotinic acid is indicated

## METABOLISM OF TRYPTOPHAN

Investigations have revealed a disturbance of tryptophan metabolism manifested by a grossly elevated excretion of urinary indican (indoxyl sulfate) and a high but variable excretion of indolylacetic acid and its conjugates in Hartnup disease In view of the relationships between tryptophan nicotinic acid and pellagra recent investigations into the primary lesion of the disease have been concerned with the metabolism of indoles It is possible that the appearance of indoles in the urine may prove to be a manifestation of faulty amino acid transport

*Biosynthesis*

Tryptophan is not synthesized by mammals and is therefore an essential dietary constituent Its biosynthesis has been studied in detail in

*Pseudomonas* [20] Previously the enzyme was called tryptophan peroxidase-oxidase because of its requirement for $H_2O_2$ Recently, Tanaka and Knox [21] have demonstrated that $H_2O_2$ is not directly involved in the reaction but is required only in catalytic amounts for activation of the iron porphyrin portion of the enzyme In addition Hayaishi et al [22] have found that atmospheric $O_2^{18}$ is incorporated into the formyl kynurenine, and not $H_2O_2^{18}$ Considerable interest has been shown in tryptophan pyrrolase because of the ease with which increased levels of this enzyme may be induced by tryptophan administration as was originally demonstrated by Knox and Mehler [23]

Kynurenine The formylkynurenine produced by tryptophan pyrrolase is hydrolyzed to formic acid and kynurenine by the enzyme formylase which is also present in liver [18 19] Kynurenine may then take part in the three pathways shown in Fig 43 2 The conversion of kynurenine to 3 hydroxykynurenine occurs in kidney and liver mitochondria in the presence of TPNH and $O_2$ [24] Atmospheric $O_2$ is incorporated into the hydroxyl group in this reaction [25] Both kynurenine and hydroxy kynurenine are cleaved by the enzyme kynureninase to alanine plus anthranilic or 3-hydroxyanthranilic acid respectively Because of the pyridoxal phosphate requirement of this enzyme [26] the metabolism of both kynurenine and hydroxykynurenine is altered in pyridoxine deficiency and appears to be shunted through the kynurenine transaminase reaction with the formation of the corresponding $\alpha$ keto acids These cyclize spontaneously to kynurenic and xanthurenic acids as is evidenced by the finding in the urine of pyridoxine deficient animals of large amounts of kynurenine kynurenic acid and xanthurenic acid as well as other tryptophan derivatives in which the side chain is still intact [27]

Anthranilic acid is apparently not formed under normal circumstances and administered anthranilic acid is excreted unchanged or as various conjugates [28] Hydroxyanthranilic acid on the other hand as already stated [17] is very active metabolically When $C^{14}$ labeled 3 hydroxy anthranilic acid is injected into rats the activity appears rapidly as $CO_2$ and as much smaller amounts of quinolinic acid N methylnicotinamide and picolinic acid The steps by which these conversions are thought to take place involve the cleavage of the benzene ring between carbons 3 and 4 with the formation of an aldehyde intermediate which has been isolated by Wiss et al [29 30] It is thought that enzymatic ring closure and decarboxylation occur simultaneously with the formation of either nicotinic acid or picolinic acid each by its specific enzyme Uncertainty remains as to whether quinolinic acid is an intermediate in these reactions Hankes and Segel who injected tritium labeled quinolinic acid into rats demonstrated the conversion of this compound to urinary N methyl nicotinamide [31] However Mehler [32] in studying the enzyme picolinic carboxylase which converts the oxidized intermediate of hydroxy

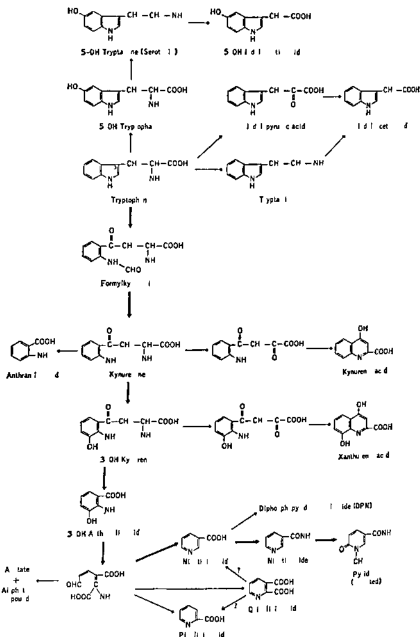



Fig 43-2 Metabolic pathways which are open to tryptophan Main pathways are shown in bold arrows

# METABOLIC ABNORMALITIES

## AMINO ACIDS

*Aminoaciduria*

The pattern of the aminoaciduria in Hartnup disease is more significant than the total amount of amino acids excreted so that chromatography must be used in the diagnosis The amounts of amino acid excreted vary from case to case and may be slightly affected by diet but are usually increased at least tenfold over the normal This absolute amount of amino acids excreted is comparable to that sometimes reached in other types of aminoaciduria such as Fanconi syndrome (see Chap 37) Table 43 2

TABLE 43-2 AMINO ACID EXCRETION IN HARTNUP DISEASE

| *Pat nt* | *Weight kg* | *C du* | *L y t H t p d ea /d y* | *N m l y t at s m g /d y* | *Meth d ( f nc )* |
|---|---|---|---|---|---|
| P H | 45 | Sy pto f | 6 6 g t t l d | | |
| | | | 150 mg tot l m d/kg | 10–20 mg t t l m d/kg | I h g [46] |
| F H | 32 | Afte tt k | 180 mg tot l m d/kg | 10–20 mg t t l d/kg | [46] |
| E H | 32 | D n g tt k | 6 0 mg m N | 41 mg N | C l nm try [1] |
| G dr a B | 24 | Sympto f | 194 mg m d N | 30 mg m d N | Gasom t y [8] |
| | | | 490 t q l t | 34 t q l t | Cl m t g pl y [8] |

shows some reported values The amino acid index (percentage of total urinary nitrogen contributed by α amino acid nitrogen) is five to seven times as high as normal

The amino acid pattern as disclosed by paper and ion-exchange chromatography is quite unlike that in any other type of generalized aminoaciduria (Fig 43 3) The striking similarity of the findings in urine specimens from different patients is apparent in Fig 43 1 The amino acid pattern found in Hartnup disease is compared with that from a normal individual in Fig 43 3, Fig 43-4 shows how it appears in two different solvent systems

The free amino acids which are excreted in amounts five to ten times normal are alanine serine threonine asparagine glutamine valine leucine isoleucine phenylalanine tyrosine tryptophan histidine and citrulline Substances which can be detected by chromatography but which are present in normal or only moderately increased amounts are taurine glycine cystine aspartic acid glutamic acid lysine methyl histidine and β amino isobutyric acid Even the most sensitive reagents

anthranilic acid to picolinic acid, found that quinolinic acid is not decarboxylated

**Nicotinic Acid** The enzyme system converting the oxidized intermediate of 3-hydroxyanthranilic acid to nicotinic acid has not yet been studied in detail Mehler et al [33] have shown that the relative amounts of picolinic acid and nicotinic acid formed from hydroxyanthranilic acid depend on the relative activity of these two enzymes In the alloxan diabetic rat the activity of the enzyme picolinic carboxylase is considerably increased This favors a production of picolinic acid rather than nicotinic acid and accounts nicely at an enzymatic level for the decreased excretion of N methylnicotinamide in this animal

From a quantitative point of view very little tryptophan appears to be converted to nicotinic acid in the normal state The dietary equivalent of 1 mg niacin is approximately 60 mg tryptophan [34]

**Serotonin** Under normal conditions the conversion of tryptophan to 5 hydroxytryptamine (serotonin) and 5 hydroxyindoleacetic acid are minor pathways In patients with malignant carcinoid however large amounts of 5-hydroxytryptamine and 5 hydroxyindoleacetic acid are excreted in the urine [35] and the blood level of serotonin is much increased over the normal [36] It seems clear that serotonin is produced by the decarboxylation of 5-hydroxytryptophan rather than by hydroxylation of tryptamine [37 38] The site of formation of 5-hydroxytryptophan is not known although the argentaffine cells have been proposed as a likely site [39] The enzyme 5 hydroxytryptophan decarboxylase is widely distributed and requires pyridoxal phosphate [40] Weissbach et al have demonstrated that 5 hydroxytryptamine is converted to 5 hydroxyindoleacetic acid by way of the intermediate 5 hydroxyindoleacetaldehyde [41]

**Indoles** Another pathway of tryptophan metabolism is conversion to indolic acids Armstrong et al have shown that normal urine contains a large number of indolic acids the main ones of which are indoleacetic acid indolelactic acid 5 hydroxyindoleacetic acid and indoleacetyl glutamine [42] Studies by Weissbach et al [43] have indicated that the indoleacetic acid excreted is a product of the metabolism of both intestinal microorganisms and mammalian tissues This conversion occurs mainly by transamination of tryptophan to indolepyruvic acid with subsequent decarboxylation to indoleacetic acid Small amounts of tryptophan are also converted to indoleacetic acid by way of tryptamine

Another important product of the metabolism of tryptophan by the intestinal microorganisms is indole which is formed from the splitting of tryptophan to indole and pyruvic acid by the enzyme tryptophanase [44] Indole absorbed from the intestine is hydroxylated in the 3 position to form indoxyl and is then conjugated primarily with sulfate This detoxication occurs mainly in the liver [45]

These excretion studies indicate a highly specific disturbance of the renal tubular reabsorption of certain amino acids No other renal ab normalities have been detected Other clearance measurements and ✓ tests of tubular function have given perfectly normal results [1] No reducing sugars are found The mother of the Hartnup family showed no

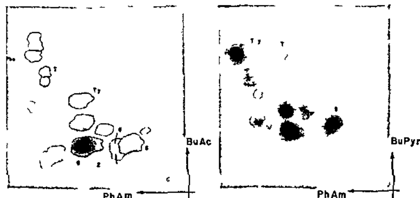



Fig 43-4 Chromatogram of a urine sample from a patient with Hartnup disease in two different solvent systems On the left the second chromatographic system was butanol-acetic acid water and on the right the second chromatogram was a butanol pyridine water system In each case the first chromatographic solvent system was phenol ammonia The urine was desalted

evidence of an abnormal amino acid clearance even in response to an oral casein load

Although the defect in renal reabsorption is easy to demonstrate this does not mean that it is necessarily the primary or only cause of the func tional disturbances In current opinion based on the work of Milne [49]

TABLE 43-3 RENAL CLEARANCE OF AMINO ACIDS IN HARTNUP DISEASE

| Amino acid | *Excretion in Hartnup disease* | *Clearance ml per min* | |
|---|---|---|---|
| | | *Hartnup* | *Normal* |
| Total amino acid N | | | |
| Fasting | High | 6–22 | 1 5 |
| Maximum on oral protein load | Very high | 25–50 | 3 0 |
| Threonine | High | 70 | 1 0 |
| Tyrosine | High | 66 | 1 5 |
| Histidine | High | 122 | 6 0 |
| Taurine | Low | 1 9 | 5 0 |
| Proline | Low | 0 3 | 0 1 |
| Cystine | Low | 0 4 | 0 5 |
| Lysine | Low | 3 0 | 0 4 |

have failed to detect significant amounts of proline, hydroxyproline methionine or arginine The failure of Evered [46] to detect tryptophan in Hartnup urine is probably due to the destruction of this amino acid on cation exchange resins

Perhaps the most striking differences between the Hartnup pattern and those of other *generalized aminoacidurias such as Fanconi syndrome* (Chap 37), Wilson s disease (Chap 25) and galactosemia (Chap 7) is the absence of an abnormality in proline excretion The quartet of amino acids found in cystinuria (viz, cystine, lysine, arginine, and ornithine, cf Chap 42) is excreted normally in Hartnup disease The free amino acids are all of the L-configuration [47]

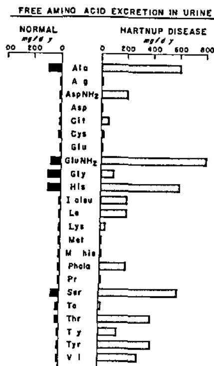



Fig 43 3 The excretion of free amino acids in the urine of normal subjects and patients with Hartnup disease

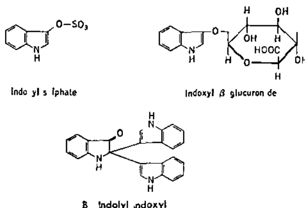
The excretion of free glutamic acid and aspartic acid is low (as in normal urine) but that of the respective amides is very high If the urine is left at room temperature for a few hours the level of free glutamic acid rises at the expense of glutamine but specimens can be kept deep frozen for years without deterioration

Acid hydrolysis of the urine releases no new amino acids and *relatively* little additional amino acid nitrogen although increased excretion of certain conjugates (e g hippuric acid and indolylacetyl glutamine) undoubtedly occurs

The aminoaciduria is of *renal* origin and not an overflow phenomenon The renal clearance of total amino acid nitrogen both in the fasting state and following an oral load of casein is very much higher than normal [48] Similarly the renal clearances of those individual amino acids which are excreted in excess are grossly elevated above normal although the clearances of the amino acids which are not excreted in excess are but little elevated [46] Table 43 3 gives some examples from the work of Dent [48] and Evered [46] The high clearance of 122 ml per min for free histidine a figure now exceeded by the 140 ml per min in a case reported by Jonxis [7] approximates the normal glomerular filtration rate and suggests a complete lack of reabsorption

detectable, even when L-tryptophan was fed in addition [1] A similar result was obtained using neomycin [54] but succinyl sulfathiazole for 7 days had no effect [4 56] These antibiotics did not alter the urinary amino acid excretion and the indicanuria returned to its high level a few days after the cessation of treatment

The indican could be raised considerably by oral administration of protein or L-tryptophan [1] Milne [49] has followed this process with chromatograms of serial samples of urine In normal subjects there was a variable rise in indican followed by a logarithmic fall but in Hartnup




Fg 43-5 The ı doxyl compounds found in the u ine of patients with Hartnup disea e

disease there was a greatly raised excretion which reached a maximum after 12 hr and persisted for more than 24 hr

The urinary indoxyl derivatives presumably are final excretory products formed in the liver from indole absorbed from the gut where it arises from the action of intestinal microorganisms on unabsorbed tryptophan The magnitude of this process in Hartnup disease could be due either to an increased growth in the gut of a particular flora or to delayed absorption of tryptophan or to both for one might determine the other There is little or no excretion of 6 hydroxyskatole sulfate the product of skatole formation characteristic of the malabsorption syndrome [57 58] Hence the responsible microorganisms may be different in Hartnup disease

Rodnight and McIlwain [4] suggested that the "pellagra of their case was caused by the diversion of tryptophan from its normal metabolic course into the wasteful pathway of indican formation This is not the only drain of tryptophan however there is not only the variable loss as indican (representing 25 per cent of the day s tryptophan intake) but also the loss as free amino acid and derived indolic acids as well

(see below) it is only one manifestation of a wider derangement of amino acid transport in this disease

*Plasma Amino Acids*

The amino acid concentrations, as determined by ion exchange chromatography [46] are about 30 per cent lower than normal In the case of tryptophan, this has been confirmed by spectrophotofluorometry [50] The following values were found for free tryptophan in plasma from three of the Hartnup boys

| *Subject* | *Age yr* | *Aminoaciduria* | *Plasma tryptophan µg/ml* |
|---|---|---|---|
| Jh H | 14 | + | 6 2 |
| H H | 12 | + | 7 8 |
| Jh H | 11 | – | 10 0 |

Westall [52] has reported that the plasma tryptophan level in normal children below 3 years of age is low, but above this age a very constant figure of 11 ± 1 µg per ml is found

METABOLITES OF TRYPTOPHAN AND INDOLE

Early in the investigations of the Hartnup family [1], a high but variable urinary excretion of indigogenic material (indican) was apparent This led to a more complete investigation of urinary indoles by paper chromatography A raised excretion of the indolic acids derived from tryptophan was thus revealed but this has not proved to be so constant a feature of the disease as was first thought

*Indicanuria*

All individuals with "Hartnup" aminoaciduria also excrete large amounts of indicans The reverse is not true For example patients with "blind loop syndrome" excrete much greater quantities of indicans but do not develop aminoaciduria [51] The urinary indican from individuals with Hartnup disease is almost entirely indoxyl sulfate (Fig 43-5), but a small amount of indoxyl β glucuronide (Fig 43 5) can accompany it [5, 51] Another indoxyl derivative (although not indigogenic) found in Hartnup urine is bis-indolyl indoxyl (Fig 43 5) which is invariably produced during enzymic oxidation of indole [53 54]

The only accurate measurements of the indican excretion in Hartnup disease are those of Rodnight [4 5] His cases excreted 200 to 400 mg per day compared with 100 or less in normal subjects of the same age Semiquantitative measurements based on chromatographic comparisons showed that indican excretion fell rapidly during a course of chlortetracycline After only 4 days on the antibiotic indican was no longer

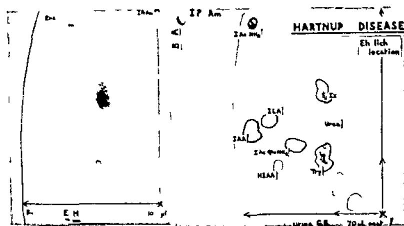



Fig 43-7 Urinary indole patterns in Hartnup disease *Left* Paper chromatogram of urine from patient E H [1] 100 μl urine were chromatographed with ascending solvents isopropanol ammonia first and up butanol acetic acid second right to left Compounds were located with p-dimethylamino benzaldehyde (Ehrlich reagent) The key is found in the right hand photograph Note the central urea spot (normally yellow) the indican spot (*Ix S*) (brown) and the triad of spots due to indolylacetic acid (*IAA*) indolylacetyl glutamine and tryptophan This pattern is typical of Hartnup disease although the relative intensity of spots is variable *Right* Paper chromatogram of urine from patient G B [8] This chromatogram shows an additional spot due to indolyllactic acid (*ILA*) not found in other cases of Hartnup disease (*By permission of D N Baron et al* [1] *and J H Jonxis* [7])

total IAA were 2 7 and 1 5 mg respectively a normal sister excreted 9 3 mg The Hartnup boy (E H ) who had the very high IAA excretion during 1951–1954 had a normal IAA excretion when measured in 1959 when he was 20 years old In 1957 colorimetric determinations of free and combined IAA [43] in urines from the other Hartnup boys gave the following results [51]

| *Subject* | *Age y* | *Aminoaciduria* | *Free IAA* | *Conjugated IAA* |
|---|---|---|---|---|
| J H | 14 | + | 15 | 5 |
| H H | 12 | + | 45 | 25 |
| Ph H | 11 | − | 2 | 2 |

It appears that the raised IAA excretion which has been found in all cases of Hartnup disease tested before the age of 12 gradually disappears as the patient grows older although the high amino acid excretion remains

## *Effect of Tryptophan Loading*

If patients with Hartnup disease are given an oral load of tryptophan a grossly abnormal excretion of indolic acids follows [1] even if there was

*Indolylacetic Acid and Its Congeners*

Paper chromatographic studies revealed that E H the first of the Hartnup family investigated, excreted indolylacetic acid (IAA) (Fig 43 6A) at a considerably higher level than normal [1] This was particularly true during his acute bouts of ataxia and rash, but even after clinical recovery the urinary abnormality was present The IAA was always accompanied by a conjugation product, originally called U 1 [59] but later identified as indolylacetyl L-glutamine (IAc GluNH$_2$) (Fig 43 6B) [60] This conjugation product is the one expected from the liver "detoxication" of IAA in man The other possible conjugate

(A) R = —CH$_2$—COOH
(B) —CH$_2$—CO—NH—CH(COOH)—CH$_2$—CH$_2$—CONH$_2$
(C) —CH$_2$—CO—O—C$_6$H$_8$O$_4$—COOH
(D) —CH$_2$—CHOH—COOH
(E) —CH=CH—CO—NH—CH$_2$—COOH

Fig 43-6 Derivatives of indole found in normal subjects and patients with Hartnup disease See text

indolylacetyl glucuronide (Fig 43 6C) [61], has been present occasionally but only in moderate amount [8]

These indoles after two dimensional paper chromatography and location with Ehrlich reagent [59 62] showed a distribution pattern quite as characteristic as the amino acid pattern (Fig 43 7) Similar indolic patterns were found with urine from the other affected members of the Hartnup family the unaffected members had normal (barely detectable) excretion of indolic acid During one period of hospitalization the free IAA excretion in E H varied from 50 to 200 mg per day on a normal varied diet A normal excretion would be less than 10 mg per day

The constancy of the findings reported above strengthened by the discovery of similar (but variable) indolic acid excretions in some other cases [1 6] has been challenged by three later findings

1 In one case of Hartnup disease [8] the urinary IAA (68 mg per day) was accompanied by indolyllactic acid (ILA) (Fig 43 6D) in an amount approaching that found in phenylketonuric patients (Chap 10) although no ortho or *p* hydroxyphenyllactic acid accompanied it [63] (Fig 43 7) ILA has not been detected in any other case of Hartnup disease

2 The excretion of IAA as of other weak acids is influenced by the urinary pH being promoted by mild alkalosis and reduced by acidosis [49] Measurements are not strictly comparable unless made under similar acid base conditions

3 Two female siblings aged 23 and 24 investigated by Hersov and Rodnight [5] had *no* elevated excretion of IAA Their daily excretions of

The whole blood and serum levels for serotonin (5-hydroxytryptamine) were on the low side of the normal range in parallel with the low urinary 5-HT and 5-HIA excretions [5 51] This may represent a slight diversion of tryptophan from serotonin formation

*Nicotinic Acid Intermediates*

Because of the pellagra like symptoms of Hartnup disease a possible enzymic block in the usual tryptophan kynurenine nicotinic acid path way (Fig 43-2) was early suspected [1] In one patient on a normal diet the urinary excretion of nicotinic acid and its derivatives was on the low side of normal and was surprisingly little elevated by oral administration of tryptophan [1] The same was true of the excretion of kynurenine as seen chromatographically [51] and of kynurenic acid and xanthurenic acid in other cases [5]

Milne [49] has now compared the rise in urinary kynurenine which follows tryptophan ingestion in normal persons and in patients with Hartnup disease Normal persons excreted 5 to 10 per cent of oral L-tryp tophan as kynurenine but in Hartnup disease the conversion was only 0 5 to 1 5 per cent

*Other Biochemical Investigations*

Considering the magnitude of the Hartnup amino acid lesion it is remarkable how normal the other biochemical and physiologic processes appear to remain Two studies bear on the part played by microorganisms in the metabolic defects In normal subjects the gut organisms are probably responsible for the production of many of the phenolic acids excreted The urinary pattern of phenolic acids in Hartnup patients receiving a normal diet is normal [13] Investigations of the fecal por phyrins in E Hartnup [1 62] disclosed that these were generally high but that they varied in a surprising way For example on one occasion 3 days of oral chlortetracycline lowered the protoporphyrin content of the feces from 74 to 11 μg per gm while the same antibiotic given 1 year later *raised* the fecal protoporphyrin more than tenfold

## METABOLIC LESION OF HARTNUP DISEASE

One might hope that all the clinical and biochemical features of Hart nup disease could be unified under a single metabolic block The following biochemical findings would have to be rationalized

1 The specific aminoaciduria
2 The retarded absorption of tryptophan from the gut
3 The raised production of IAA which probably diminishes with the age of the subject

normal excretion of IAA before the load [49]. In normal subjects 70 mg per kg L-tryptophan causes a sharp rise in IAA and a smaller rise in ILA. The peak is reached after 2 hr and the level falls to normal values in 12 hr. In Hartnup disease the rise is somewhat higher and persists at a high level for at least 24 hr. Residual free tryptophan may appear in the feces. If DL-tryptophan is fed to normal subjects, ILA appears in the urine in large quantities. It is presumably formed from absorbed D-tryptophan by the action of D-amino acid oxidase.

Hartnup patients react quite differently. There is little excretion of ILA. Most of the D-tryptophan is recovered from the feces. Milne's conclusion from these experiments [49] is that there is a defective absorption of L- and of D-tryptophan from the jejunum. The absorption block for *D-tryptophan is thought to be virtually complete, the partial block for* L-tryptophan would prolong its oxidative metabolism to IAA, but whether this occurs in the tissues or in the gut must await the results of studies after administration of antibiotics. The excretion of IAA by normal subjects is only partially suppressed by neomycin or tetracycline [43] and in one Hartnup patient was actually raised by chlortetracycline [1] presumably because tryptophan was no longer diverted to indican.

*Other Urinary Indoles*

Another indolic acid which has been found in urine of patients with Hartnup disease is indolylacryloyl glycine (Fig. 43.6E). This is the expected conjugation product of indolyl acrylic acid. This compound which reacts atypically with Ehrlich reagent, was first described by Kimmig [64] in urine from patients with light sensitive dermatitis. It is excreted by man after the ingestion of indolylpropionic acid. Urine from a Hartnup patient on a normal diet contains much more indolylacryloyl glycine than does normal urine [51] and its excretion is stimulated after an 8 hr lag period by oral administration of tryptophan [49]. Chromatographic methods have failed to show indolylpyruvic acid or its decomposition products in urine [55, 61, 65].

The urinary excretion of tryptamine in Hartnup patients is normal [5]. This may only be tryptamine produced locally in the kidney tubules and gives no indication of tryptamine formation in other tissues.

*Indolic Acids in the Blood*

The whole blood and plasma indolic acids have been studied chromatographically in some cases of Hartnup disease [5, 51]. Apart from a slightly elevated concentration of indoxyl sulfate, patients and normal subjects were indistinguishable. The renal clearance values for indolic acids are normally very high [66] and there is no evidence that they are raised higher in the disease. The clearance of IAcGluNH$_2$ is at least 150 ml per min and may be synthesized at least in part from IAA in the kidney

lead to an increased metabolism along alternative pathways to indolyl pyruvic acid or tryptamine and thence to IAA It is noteworthy that the quite different shunt of tryptophan metabolism in carcinoidosis also leads to pellagra like rashes

The other biochemical abnormalities would be considered consequences of endogenous nicotinic acid deficiency at the cellular level For example the aminoaciduria would be a result of cessation of tubular reabsorption consequent to the nicotinic acid deficiency

It is unlikely that IAA has toxic consequences Consumption in vast quantities as an insulinase inhibitor [72] does not cause aminoaciduria to develop [51] but IAA does have important effects on membrane permeability and amino acid uptake in plant cells

*Abnormal Intestinal Bacteria*

It seems improbable that the primary abnormality of Hartnup disease lies in an unusual type or concentration of microorganisms It is much more likely that a tissue dysfunction promotes the growth of an unusual flora Thus retention or secretion into the gut of amino acids or indoles could provide an environment favorable for abnormal flora It is known that the oral administration of methionine causes distinct changes in the urinary indole chromatograms from Hartnup patients [56]

*Amino Acid Transport*

The most acceptable hypothesis for the primary lesion in Hartnup disease is that the transport of amino acids into cells is defective [49] The defect would have to involve at least the cells of the proximal renal tubules and of the jejunum in order to account for the aminoaciduria and the retention of tryptophan in the lumen of the gut It is not yet known whether those amino acids which are poorly reabsorbed by the renal tubule are also poorly absorbed from the gut nor why some amino acids do not appear in Hartnup urine at all

It has recently been suggested by Dickinson [73] that xanthinuria (Chap 22) is an example of a renal defect of purine reabsorption chemically coupled with a specific xanthine oxidase deficiency a possibility already predicted by Harris [74] Hartnup disease may be another example i e the specific defect of tryptophan pyrrolase (oxidase) deficiency associated through some unknown common factor with a less specific deficiency of amino acid transport in renal and intestinal cells

Whatever the ultimate cause of Hartnup disease may prove to be the abnormal metabolism of tryptophan may only reflect the experimental ease with which its degradation products can be measured There is of course considerable diversion of tryptophan from its normal metabolic

4 The low experimental conversion of oral tryptophan along the nicotinic acid pathway

5 The harboring of gut microorganisms with a special aptitude for the conversion of tryptophan to indican

Three possibilities for the primary lesion are immediately obvious and were discussed in the definitive paper [1]

1 A block along the metabolic sequence from tryptophan through kynurenine to nicotinic acid

2 An intestinal habitat which supports a bacterial flora so abnormal that the metabolism of the host is affected

3 A disorder of amino acid transport in the cells of the kidney tubules and in the gut

*Tryptophan Metabolism and Nicotinic Acid*

A considerable fraction of dietary tryptophan is normally available for the synthesis of nicotinic acid It is possible that some tissue systems obtain their required nicotinic acid preferentially (or only) from the *in situ* metabolism of tryptophan rather than from dietary nicotinic acid Since the conversion of dietary tryptophan to kynurenine and nicotinic acid derivatives is greatly impaired in Hartnup disease it is possible that an endogenous ' nicotinic acid deficiency could develop One might then account for the signs of pellagra especially under conditions of growth or stress Nicotinamide deficiency leads to mental deterioration [68]

A genetically determined metabolic block most probably would involve tryptophan pyrrolase an enzyme which normally catalyzes the oxidation of tryptophan to formylkynurenine [21] This is an adaptive enzyme which is absent in fetal tissue but develops dramatically after birth If this is the deficient enzyme in Hartnup disease the condition is a carry over of the fetal condition into postnatal life [69] The clinical improvement in adult life might represent a gradual adaptive appearance of tryptophan pyrrolase If the substrate adaptation ' feature of the *enzyme really is a stabilization by tryptophan [70]* then the Hartnup enzyme would have an abnormal lability On the other hand there is yet no proof of an actual deficiency of enzyme and the apparent deficiency might equally well be a reflection of reduced availability of tryptophan

Snyder [71] has considered that Hartnup disease is due to dysfunction of an apoenzyme requiring nicotinamide as a cofactor but there is little evidence in support of this view

The shunting of tryptophan from nicotinic acid formation would

Hartnup disease should be suspected in patients with the following signs pellagra not due to gross dietary deficiency photosensitive rash especially if accompanied by neurologic changes reversible ataxia especially when siblings are affected similarly a high excretion of indican or indolylacetic acid

## GENETICS

The 13 known cases (4 males 9 females) are distributed among 8 families with a total of 32 children (Table 43 1) Siblings are affected in 3 families 4 out of 8 in one family [1] 2 out of 3 in another [5] and 2 out of 9 in the third [7] In 2 of these families the parents are first or second cousins 1 or 4 other families [5 6 8] no blood relationship between parents could be established even back to 1416 in the last case! Five of the families come from England 2 from Holland and 1 from Germany but no ethnic pattern is discernible No cases have been reported in the United States

Obviously these figures are insufficient to warrant conclusions about *either mode of transmission or prevalence of the disease If the disease* is genetically determined it swells the list of those hereditary abnormalities which are not apparent at an early age but appear only under the stimulus of some external or internal metabolic influence Although, the disease appears to be rare it may be that the rarity resides in the appropriate or adequate stress rather than in the genetic existence of susceptibility

A possibility remains that an abnormal gut flora is solely responsible for the manifestations of Hartnup disease If this should prove to be true the condition could still be called hereditary provided that the abnormal flora were encouraged by a genetically determined vagary of the host [1]

If Hartnup disease is transmitted as an autosomal recessive trait as the *original pedigree suggests affected individuals would be homozygous* for the condition and both their parents would be heterozygous carriers It might be expected that heterozygotes would show a less marked expression of the condition at the site of the primary metabolic defect Perhaps this could be used to show which of several alternative metabolic lesions is the most likely [60]

Most of the parents and near relatives of Hartnup patients have been tested for aminoaciduria and all are uniformly negative [6] The mother of the Hartnup children had a *normal amino acid clearance* even in response to a casein load [48] but a single experiment [51] suggested that she responded to an oral load of L-tryptophan with a higher than normal excretion of indolylacetic acid and a lower than normal conversion to nicotinic acid intermediates Two of the affected girls are married but

routes and it is reasonable to suggest that the clinical aspects of Hartnup disease may result from one or more of the following

1 Lowered production of endogenous nicotinic acid
2 Increased absorption of indole from the gut
3 Tissue or gut formation of tryptamine [75]
4 Chronically low level of plasma tryptophan

## DIAGNOSIS

The only constant feature of Hartnup disease is the characteristic excretion of free amino acids and it is upon this that diagnosis must be based The *pattern* of excreted amino acids, rather than the total amino acid excretion is the determining factor It is not sufficient to measure total amino acid nitrogen even though a low total value would exclude the disease

Any of the simple two dimensional paper chromatographic systems and location agents for amino acids [76] will serve Figures 43 3 and 43-4 show the urinary amino acids expected in Hartnup disease [1 to 8] A volume of urine corresponding to a 2 sec excretion from a 24 hr collection on a normal or low protein diet will give intensely reacting spots with the ninhydrin reagent Normal urine shows very little Desalting is hardly necessary with this small volume The Hartnup amino acid pattern has never changed in any of the patients during the years they have been studied Patients show only the most minor variation in pattern and nothing like it has been found in any other condition

The only alternative diagnosis for Hartnup disease would be pellagra, and in the few cases of true dietary pellagra examined [1, 51] the amino acid excretion was low or normal

The indolic excretion is not constant enough to be the basis of a diagnostic test but the abnormal response to oral L-tryptophan loading accompanied by paper chromatography of serial samples might be used [49] Hartnup disease would be associated with a greater than normal excretion of indolylacetic acid and of indican persisting for 24 hr at least A semiquantitative test tube method is suggestive e g the Obermeyer test for indican or the Weissbach test [77] for indolic acids

Mix 1 ml urine 2 drops concentrated HCl and 1 ml CHCl shake well centrifuge and reject aqueous layer mix with 1 ml 0 5 per cent Ehrlich reagent in 12 N HCl wait 10 min mix with 0 5 ml ethanol add 1 drop 2 5 per cent $NaNO_2$ An immediate blue color indicates urinary indolic acids at a level above 50 µg per ml

Hartnup disease is not excluded by a normal indole excretion but all patients with a high indican or high IAA excretion [43] should be further examined for the Hartnup amino acid excretion pattern

c A disorder of amino acid transport into cells particularly those of the renal tubules and the jejunum accounts for the constant aminoaciduria and for the slowed absorption of tryptophan The latter permits an abnormally large formation in the gut of indicans and may also sponsor the appearance of an unusual intestinal flora The clinical manifestations arise through the consequent deviation of dietary tryptophan from its normal metabolic routes especially that leading to nicotinic acid

The evidence favors the third interpretation of the abnormality of Hartnup s disease

6 Insufficient number of patients with this disease has come to attention to permit certainty regarding its inheritance pattern but information at present is consistent with autosomal recessive transmission

7 Patients have responded satisfactorily to oral administration of nicotinamide A diet high in protein may be of value as well

## BIBLIOGRAPHY


1 Baron D N Dent C E Harris H Hart E W and Jepson J B Hereditary pellagra like skin rash with temporary cerebellar ataxia constant renal amino aciduria and other bizarre biochemical features Lancet 2 421 1956
2 Hickish G W Pellagra in an English child Arch Dis Childhood 30 195 1955
3 Hersov L A A case of childhood pellagra with psychosis J Ment Sc 101 878 1955
4 Rodnight R and McIlwain H Indicanuria and the psychosis of a pellagrin J Ment Sc 101 884 1955
5 Hersov L A and Rodnight R Hartnup disease in psychiatric practice clinical and biochemical features of three cases J Neurol Neurosurg & Psychiat (in press)
6 Henderson W A case of Hartnup disease Arch Dis Childhood 33 114 1958
7 Jonxis J H Oligophrenia phenylpyruvica en de Hartnupziekte Nederl tijdschr geneesk 101 569 1957
8 Weyers H and Bickel H Photodermatose mit Aminoacidurie Indolacturie und cerebralen Manifestationen (Hartnup Syndrom) Klin Wchnschr 36 893 1958
9 Yanofsky C Enzymatic studies with a series of tryptophan auxotrophs of *Escherichia coli* J Biol Chem 224 783 1957
10 Doy C H and Gibson F 1 (o-carboxyphenylamino) 1-deoxyribulose A compound formed by mutant strains of *Aerobacter aerogenes* and *Escherichia coli* blocked in the biosynthesis of tryptophan Biochem J 72 586 1959
11 Yanofsky C and Rachmeler M The exclusion of free indole as an intermediate in the biosynthesis of tryptophan in *Neurospora crassa* Biochim et biophys acta 28 640 1958
12 Parks L W and Douglas H C N fructosyl anthranilic acid as a possible intermediate in the synthesis of indole by *Saccharomyces* Biochim et biophys acta 23 207 1957
13 Umbreit W W Wood W A and Gunsalus I C The activity of pyridoxal phosphate in tryptophane formation by cell free enzyme preparations J Biol Chem 165 31 1946
14 Dalgliesh C E and Tabechian H Comparison of the metabolism of uniformly C-labeled L-phenylalanine L-tyrosine and L-tryptophan in the rat Biochem J 62 625 1956

neither has children Further elucidation must await the discovery of many more cases and of a test for genetic carriers of the condition

## TREATMENT

The paucity of cases and their variability has not made it easy to evaluate possible courses of treatment Similarity of the symptoms to pellagra has led in many instances to oral nicotinamide therapy (40 to 200 mg amide or acid per day) Marked improvement in the dermatitis and neurologic picture has usually followed, but such improvement may occur without treatment In view of the high urinary loss of amino acids a high protein diet or supplement has been prescribed and seems beneficial

## SUMMARY

1 Hartnup disease is characterized by an intermittent red scaly, pellagra like rash appearing after exposure to sunlight attacks of cerebellar ataxia and occasionally by psychiatric changes ranging from emotional instability to delirium Progressive mental deterioration was suggested from the original study of the Hartnup family but this has not been supported by patients subsequently encountered

2 The disease was originally observed in 4 out of 8 offspring of a first cousin marriage Further patients, including 3 more sets of siblings have brought the total of known cases to 13

3 The single recognized constant feature of the disease is a massive aminoaciduria renal in origin and characteristic in pattern This provides the only certain diagnostic test

4 All patients excrete large but variable amounts of indoxyl sulfate (indican) in the urine This disappears if the gut is sterilized with antibiotics Also in most cases there are abnormally large amounts of indolyl acetic acid and its conjugates Following oral administration of a loading amount of L-tryptophan there is a high and prolonged conversion to urinary indolylacetic acid coupled with a lower than normal conversion to kynurenine and nicotinamide

5 *Three possibilities have been considered for the primary* genetically determined biochemical lesion

*a* An enzymic block along the metabolic sequence from tryptophan through kynurenine to nicotinic acid with necessary diversion of tryptophan degradation The aminoaciduria the abnormal gut flora and the clinical symptoms develop because of a deficiency in the intrinsic production of nicotinic acid

*b* An abnormal intestinal environment supports a gut flora which deranges the metabolism of the host

38 Udenfriend S Creveling C R Posner H Redfield B G Daly J and Witkop B On the ability of tryptamine to serve as a precursor of serotonin Arch Biochem & Biophys 83 501 1959
39 Dalgliesh C E and Dutton R W On the site of formation of 5-hydroxy tryptophan Biochem J 65 21P 1957
40 Clark C T Weissbach H and Udenfriend S 5-hydroxytryptophan decarboxylase preparation and properties J Biol Chem 210 139 1954
41 Weissbach H Redfield B G and Udenfriend S Soluble monoamine oxidase its properties and actions on serotonin J Biol Chem 229 953 1957
42 Armstrong M D Shaw K N F Gortatowski M J and Singer H The indole acids of human urine Paper chromatography of indole acids J Biol Chem 232 17 1958
43 Weissbach H King W Sjoerdsma A and Udenfriend S Formation of indole 3-acetic acid and tryptamine in animals A method for estimation of indole 3-acetic acid in tissues J Biol Chem 234 81 1959
44 Happold F C Tryptophanase tryptophan reaction Advances Enzymol 10 51 1950
45 Stoppani A O M Precursors of indoxyl J Biol Chem 157 1 1945
46 Evered D F The excretion of aminoacids by the human—a quantitative study with ion-exchange chromatography Biochem J 62 416 1956
47 Bonetti E and Dent C E The determination of optical configuration of naturally-occurring aminoacids using specific enzymes and paper chromatography Biochem J 57 77 1954
48 Dent C E The renal aminoacidurias Exper Med & Surg 12 2?9 1954
49 Milne M D Crawford M A Girao C B and Loughridge L The metabolic abnormality of Hartnup disease Biochem J 72 301 1959
0 Duggan D E and Udenfriend S The spectrophotofluorometric determination of tryptophan in plasma J Biol Chem 223 313 1957
51 Jepson J B Unpublished observations
5? Westall R G Roitman E Iera C Rasmussen H Craviato J Gomez F and Holt L E The plasma aminoacids in malnutrition Arch Dis Childhood 33 499 1958
53 Holmes Siedle A G and Saunders B C The peroxidase-catalysed oxidation of indole Chem & Ind 265 1957
54 Loo Y H and Woolf D O Microbiological oxidation of indole Chem & Ind 1123 1957
55 Shaw K N F Unpublished observations
56 Murray W G D Unpublished observations
57 Horning E C and Dalgliesh C E The association of skatole forming bacteria in the small intestine with the malabsorption syndrome and certain anaemias Biochem J 70 13P 1958
8 Horning E C Sweeley C C Dalgliesh C E and Kelly W Mammalian hydroxylation in the 6-position of the indole ring Biochim et biophys acta 32 566 1959
9 Jepson J B Paper chromatography of urinary indoles Lancet 2 1009 1955
60 Jepson J B Indolylacetylglutamine and other indole metabolites in Hartnup disease Biochem J 64 14P 1956
61 Jepson J B Indolylacetamide a chromatographic artifact from the natural indoles indolylacetylglucosiduronic acid and indolylpyruvic acid Biochem J 69 ?1 1958
62 Jepson J B In *Chromatographic and Electrophoretic Techniques* edited by I Smith 2d ed vol 1 chap 9 Heinemann London 1960
63 Jepson J B Smith A J and Strong L B An inborn error of metabolism with urinary excretion of hydroxyacids keto acids and aminoacids Lancet 2 1334 1958
64 Jummig J Sticherling W Tschesche R and Urbach H G Indolylacryloylglycine isolierung aus Harn und Synthese Ztschr physiol Chem 311 ?34 1958
65 Kaper J M and Veldstra H On the metabolism of tryptophan by *Agrobacterium tumefaciens* Biochim et biophys acta 30 401 1958

15 Gholson R K Rao D R Henderson L M Hill R J and Koeppe R E The metabolism of DL-tryptophan 7α-$C^{14}$ by the rat J Biol Chem 230 179 1956
16 Gholson R K and Henderson L M The formation of acetate from the benzene ring of tryptophan Biochim et biophys acta 30 425 1958
17 Hankes L V and Henderson L M The metabolism of carboxyl labeled 3-hydroxy anthranilic acid in the rat J Biol Chem 225 349 1957
18 Mehler A H and Knox W E The conversion of tryptophan to kynurenine in liver I The coupled tryptophan peroxidase-oxidase system forming formyl kynurenine J Biol Chem 187 419 1950
19 Mehler A H and Knox W E The conversion of tryptophan to kynurenine in liver II The enzymatic hydrolysis of formylkynurenine J Biol Chem 187 431 1950
20 Hayaishi O and Stanier R Y The bacterial oxidation of tryptophan III Enzymatic activities of cell free extracts from bacteria employing the aromatic pathway J Bacteriol 62 691 1951
21 Tanaka T and Knox W E The nature and mechanism of the tryptophan pyrrolase (peroxidase-oxidase) reaction of *Pseudomonas* and of rat liver J Biol Chem 234 1162 1959
22 *Hayaishi O Rothberg S Mehler A H and Saito Y Studies on oxygenases* Enzymatic formation of kynurenine from tryptophan J Biol Chem 229 889 1957
23 Mehler A H and Knox W E The adaptive increase of the tryptophan peroxidase-oxidase system of liver Science 113 237 1951
24 DeCastro F T Price J M and Brown R R Reduced triphosphopyridine-nucleotide requirement for the enzymatic formation of 3 hydroxykynurenine from L-kynurenine J Am Chem Soc 78 2904 1956
25 Saito Y Hayaishi O Rothberg S and Senoh S L-kynurenine hydroxylase Fed Proc 16 240 1957
26 Braunshtein A E Goryachenkova E V and Lashkina T S Enzymic formation of alanine from L-kynurenine and L-tryptophan and the role of vitamin $B_6$ in this process Biokhimiya 14 163 1949
27 Dalgliesh C E The relation between pyridoxin and tryptophan metabolism studied in the rat Biochem J 52 3 1952
28 Mason M The metabolism of tryptophan in riboflavin-deficient *rats J Biol* Chem 201 513 1953
29 Wiss O Semmer H and Peters H Transformation of 3-hydroxyanthranilic acid to quinolinic acid and nicotinic acid in the mammalian organism I Enzymic oxidation of 3-hydroxyanthranilic acid Ztschr physiol Chem 304 221 1956
30 Wiss O and Bettendorf G The conversion of 3 hydroxy anthranilic acid to quinolinic acid and nicotinic acid in animal organisms II The isolation and preliminary characterization of the primary oxidation product of 3 hydroxy anthranilic acid Ztschr physiol Chem 306 145 1957
31 Hankes L V and Segel I H Synthesis and metabolism of quinolinic acid ring labeled with tritium Proc Soc Exper Biol & Med 94 447 1957
32 Mehler A H Formation of picolinic and quinolinic acids following enzymatic oxidation of 3 hydroxyanthranilic acid J Biol Chem 218 241 1956
33 Mehler A H McDaniel E G and Hundley J M Changes in the enzymatic composition of liver I Increase of picolinic carboxylase in diabetes J Biol *Chem 232 323 1958*
34 Goldsmith G A Niacin tryptophan relationships in man and niacin requirement Am J Clin Nutrition 6 479 1958
35 Curzon G The excretion of indoles in argentaffinoma Arch Biochem & Biophys 66 497 1957
36 Sjoerdsma A and Udenfriend S Studies on indole metabolism in patients with malignant carcinoid (Argentaffinoma) J Clin Invest 34 914 1955
37 Davidson J Sjoerdsma A Loomis L N and Udenfriend S Studies with the serotonin precursor 5-hydroxytryptophan in experimental animals *and man* J Clin Invest 36 1594 1957

Part Eleven

# Diseases Involving a Deficiency of Circulating Enzymes or Plasma Proteins

66 Despopoulos A and Weissbach H Renal metabolism of 5-hydroxy indoleacetic acid Am J Physiol 189 548 1957
67 Rimington C Unpublished observations
68 Gregory I The role of nicotinic acid (niacin) in mental health and disease J Ment Sc 101 85 1955
69 Nemeth A M and Nachmias V T Changes in tryptophan peroxidase activity in developing liver Science 128 1085 1958
70 Dubnoff J W and Dimick M The stability of the adaptive enzyme tryptophan peroxidase Biochem et biophys acta 31 541 1959
71 Snyder L H Fifty years of medical genetics Science 129 7 1959
72 Mirsky I A Insulinase insulinase inhibitors and diabetes mellitus Rec Prog Hormone Res 13 429 1957
73 Dickinson C J and Smellie J M Xanthinuria Brit M J 2 1217 1959
74 Harris H In *Modern Views on the Secretion of Urine* edited by F R Winton p 208 Churchill London 1956
75 Sjoerdsma A Oates J A Zaltzman P and Udenfriend S Identification and assay of urinary tryptamine application as an index of monoamine oxidase inhibition in man J Pharm Exper Therap 126 217 1959
76 Smith I Editor *Chromatographic and Electrophoretic Techniques* vol I chap 5 Heinemann London 1960
77 Weissbach H Unpublished observations

# Chapter 44

# Hypophosphatasia

*Frederic C Bartter*

## DEFINITION AND GENERAL DESCRIPTION

Hypophosphatasia is a familial disease characterized by abnormalities of the skeleton decreased serum alkaline phosphatase and the appearance of phosphoethanolamine in the plasma and urine Hypercalcemia renal damage and occasionally premature synostosis of the cranial vault may also be found

### HISTORICAL ASPECTS

Although the disease was first given its present name by Rathbun [1] in 1948 there are scattered reports of what is undoubtedly the same entity going back 20 years before that time The historical aspects are covered in detail in the reviews by Fraser [2] and by Currarino and associates [3] The following reports are of especial interest in the development of currently accepted concepts of the disease Huhne [4] in 1929 described a patient with a syndrome clearly recognizable in retrospect as hypophosphatasia Chown [5] in 1935 described two further patients with nephrocalcinosis at autopsy Kubatsch [6] in 1938 described a patient with islands of bone in the skull whose father had evidence of premature synostosis Alkaline phosphatase values were not reported in any of these cases Anspach [7] in 1939 noted hypercalcemia in a child with pathognomonic changes in the bones including premature synostosis Serum alkaline phosphatase concentration in this child determined on a number of occasions was very low with a single exception in early infancy Macey [8] in 1940 described two brothers (one 36 years of age) with hypophosphatasia who gave a history of severe rickets in childhood and of numerous fractures in adult life Rathbun in his classical description of the syndrome reported that alkaline phosphatase activity was low in bone intestinal mucosa and kidney Sobel

and increase in intracranial pressure may occur convulsions serious brain damage and death may ensue if surgical decompression is not per formed The late manifestations are those of oxycephaly The sutures may show the characteristic prominent bony ridges Subperiosteal new bone formation not normally seen in rickets has been reported in three cases [1, 2 17] Premature loss of teeth is occasionally seen




Fig 44-1 X ray of the shoulder (A) and wri t (B) of a 39 month-old female with hypophosphata a Note defects in epiphyseal o sification (*By permiss on of Dr Edna Sob l*)

Hypercalcemia is common especially in infants and hypercalcinuria results The nausea and vomiting of some patients may be in part at tributable to the hypercalcemia

Four ca es have been observed first as adults [8 18 19] In two brothers rickets was said to have been present up to the age of 4 followed by an interim period of apparently normal health for a number of years The patients finally came to medical attention with pseudofractures one showing evidence suggestive of craniostenosis in childhood In the third adult patient rickets had been suspected in childhood The fourth had osteoporosis hypophosphatasia and phosphoethanolaminuria found

and associates [9] in 1953 considerably advanced the knowledge of this syndrome In their patient they noted for the first time premature loss of teeth as part of the syndrome and observed that the serum did not inhibit normal phosphatase activity They found low alkaline phosphatase activity in bone, cartilage, liver and a tooth, and finally presented data strongly suggestive of hypersensitivity to vitamin D in their patient

Fraser and associates [10] and McCance and associates [11] discovered simultaneously in 1954, that the excretion of phosphoethanolamine in the urine is an integral feature of the disease They showed also that the parents of patients might have abnormally low serum alkaline phosphatase values Fraser et al [10] found that phosphoethanolamine might appear in the plasma of patients and in the urine of the otherwise normal parents of patients with hypophosphatasia Fraser [12] also showed that the cartilage of rachitic rats would calcify in the serum of patients with hypophosphatasia but that costochondral cartilage and osteoid from a patient would not calcify in the serum of normal people or in a synthetic medium containing appropriate concentrations of calcium and phosphate ions Scaglione [13] in 1956 reported that a patient with abnormally low phosphatase activity in bone might nevertheless have normal phosphatase activity in liver duodenal juice, and indeed, in a tissue culture of osteoblasts In 1958 Kretchmer and associates [14] reported alkaline phosphatase absent from the leukocytes of a patient and normal in those of the mother (whose serum phosphatase concentration was low)

## CLINICAL DEFECTS

The clinical findings in hypophosphatasia are probably all attributable to the defect in formation of true bone to premature synostosis of the skull or to the hypercalcemia which is a frequent but not constant feature Since the essential feature of the generalized bone disease is inadequacy of mature bone formation the clinical manifestations are more severe the earlier the appearance of the disorder When it appears in utero infants are often stillborn and lack adequate bony support for cranial and thoracic cavities When it appears in adult life, patients may be asymptomatic or have only symptoms attendant upon an occasional fracture The disease of bone induces the gross changes of true rickets with beading of costochondral junctions bowing of the legs and widening of the ends of long bones Figure 44 1 shows the epiphysis of a child of 39 months with hypophosphatasia The premature synostosis may be anticipated in infancy by the *absence* of radiologically demonstrable bone over large areas of skull giving the appearance of wide sutures separating plaques of bone As in the syndrome of oxycephaly or acrocephalosyndactyly [15 15a] these areas represent in fact uncalcified osteoid without fibrous septums [16] As they calcify exophthalmos

junction of a child with the disorder [9] The defect in appositional and subperiosteal bone formation appears as wide zones of uncalcified osteoid lined with osteoblasts There is virtually no osteoclasis nor is there *fibrosis of the marrow or other evidence of bone destruction* As in osteomalacia the total mass of bone plus matrix may appear greater than normal [16] The defect in membranous bone which may be so extensive as to leave only plaques of true bone in the skull [1] is histologically comparable showing wide areas of uncalcified osteoid *It* has been observed that the true bone in this disease may have a microradiologic structure relatively primitive for the patient s age and that the collagen fibrils may be correspondingly poorly arranged [20] Material from patients with true rickets of comparable degree and severity is not available for comparison It may be that the primitive structure results from the relative absence of remodeling of osteoid as compared with that in true bone The first step in remodeling is of course bone destruction which is indicated histologically by the presence of osteoclasts Osteoclasts are extremely rare in the bones during active rickets or osteomalacia

## CALCIFICATION PROCESS

It is not known how the biochemical defects (decreased serum alkaline phosphatase activity elevated plasma and urine phosphoethanolamine levels and occasionally elevated serum and urine calcium concentration) are interrelated The disorder may be better understood in the light of current concepts of bone physiology

Bone formation begins with the deposition of matrix in apposition to preexisting bone or calcified cartilage or with its formation *de novo* in intramembranous sites The osteoid is deposited by osteoblasts which contain alkaline phosphatase Some alkaline phosphatase from osteoblasts is liberated into the circulating fluids In the absence of liver disease the serum alkaline phosphatase concentration closely parallels and serves as an index of the number and activity of osteoblasts

Deposition of mineral in osteoid probably begins with the formation of an initial complex of calcium and phosphorus which in all probability is $CaHPO_4$ [21] The formation of this complex depends in part upon the activity product of $Ca^{++} \times HPO_4^{--}$ surrounding the osteoid and in part upon the structure of the osteoid itself Once formed it is spontaneously rearranged at physiologic pH to the hydroxyapatite structure of bone Experimental *evidence regarding the essential ion product has been* derived largely from studies with rachitic cartilage It is assumed without good evidence that calcification of osteoid follows the same principles Calcification *does not appear in rachitic cartilage incubated in* solutions with a solubility product below a critical figure The same applies of course to solutions of calcium phosphate alone The important

first in adult life with no evidence of earlier disease It thus appears that spontaneous, virtually complete remissions may occur and that new manifestations may not appear for a number of years

## LABORATORY DIAGNOSIS

Laboratory diagnosis consists of (1) demonstration of serum alkaline phosphatase values below normal for the patient's age (2) histologic characterization of the bone lesion, (3) demonstration of phosphoethanolamine in the urine This compound has not been identified in



Fig 44-2 Costochondral junction of a rib of a 33-month-old girl with hypophosphatasia Note disorganization of cartilage and osteoid seams lined with osteoblasts Undecalcified (*By permission of Dr Edna Sobel*)

the urine of normal subjects Quantitative data for phosphoethanolamine in urine or plasma in hypophosphatasia are not yet available

Chemical analysis may also allow the recognition of hypophosphatasia in persons with no signs or symptoms of the disease Relatives of patients may have low serum alkaline phosphatase values and phosphoethanolamine in the urine (see below) These persons presumably are heterozygous for hypophosphatasia

The histologic picture is generally indistinguishable from that of true rickets The defect in ossification from cartilage appears as in rickets, as a widening of the zones of provisional calcification disruption of the normal columnar arrangement of cells and failure of calcification of degenerating cartilage Figure 44 2 shows a section from a costochondral

phatase in the calcification process however since it has been shown that beryllium has this property in concentrations which do not affect liberation of inorganic phosphate from β glycerophosphate [27]

Finally it has been suggested that phosphatase serves to destroy phosphate esters which *inhibit* calcification as crystal poisons [28] In this view, the phosphate esters which are promoting calcification in slice experiments 'protect phosphate-containing sites from phosphatase leached from the slices [21]

## BIOCHEMICAL DEFECTS IN HYPOPHOSPHATASIA

Although rachitic cartilage (and probably the uncalcified osteoid in osteomalacia) normally calcifies readily in normal concentrations of calcium and phosphate ions this does not occur in patients with hypophosphatasia In this syndrome the serum phosphorus level is normal and the serum calcium level is frequently elevated despite the presence of histologic rickets Furthermore Fraser showed directly that rachitic cartilage would calcify in the serum of a patient with hypophosphatasia This makes it unlikely that the defect in calcification results from too low an activity product of circulating calcium and phosphate ions The possibility does exist however that calcium may be present in a complexed nonionized form This is suggested by the observation that vitamin D in average doses may rapidly elevate the serum calcium concentration of patients with hypophosphatasia [9] Anning and associates [9] showed that vitamin D can increase significantly the concentration of diffusible nonionized calcium On the other hand if calcification depends upon the activity of phosphate locally at osteoid sites and this is dependent upon phosphatase the disorder may indeed involve a decrease of effective ion product

The syndrome thus provides strong clinical evidence that alkaline phosphatase plays a role in the calcification process Since osteoid is present in abundance it appears likely that phosphatase is not required for its formation There remains the possibility that osteoid formed without alkaline phosphatase is abnormal e g lacks a phosphorus containing compound required for in vivo calcification such as the mucopolysaccharide described by DiStefano and associates [30] As noted above the rachitic cartilage of a patient with hypophosphatasia did not calcify in normal serum [12] Attempts to define further the relation ship between the enzyme deficiency and the bone disease have proved unsuccessful

An inhibitor of alkaline phosphatase could not be found in the serum of patients although carefully sought for by four groups of investigators [9 10 16 20]

point is that calcification in vivo normally occurs despite a product of activities of $Ca^{++} \times HPO_4^{--}$ in serum below that required for spontaneous precipitation (Hydroxyapatite the mineral phase of formed bone will remove calcium and phosphate from solutions with activity product $Ca^{++} \times HPO_4^{--}$ below that of serum The present problem however, concerns a failure of calcification *de novo* of newly formed osteoid or "calcifiable" cartilage )

A number of attempts have been made to explain this paradox The first attempt (which antedated indeed accurate knowledge of the appropriate activity products) was that of Robison [22] who noted the presence of alkaline phosphatase in all areas where calcification occurs He suggested that phosphatase acts to liberate inorganic phosphate at the site of calcification and thus by raising the activity product of calcium by phosphate ions locally, to initiate crystallization This view was strengthened by the observation that calcification of rachitic cartilage would not occur after inactivation of phosphatase, save at concentrations of calcium and phosphate ions far above the physiologic range [23] The concept was rendered untenable, however by the demonstration that inhibitors of glycolysis not affecting alkaline phosphatase could inhibit calcification of cartilage in concentrations of calcium and phosphate well above the physiologic range It was shown that calcification is blocked by phlorizin iodoacetate or fluoride but that it can be restored in the presence of the first two if glucose 1 phosphate or 3 phosphoglycerate respectively, is added these compounds appear "below the block in glycolysis ATP itself would not promote calcification so it does not appear that the glycolytic cycle serves solely to supply energy [24]

Further this inhibition can be overcome by the addition of substrates (such as phenylphosphate) not in the pathway of glycolysis but readily hydrolyzed by phosphatase These observations led Gutman and Yu to propose that phosphatase acts as a transferase of phosphate from organic phosphate esters to available sites on the matrix [24]

Two other theories have been proposed to explain the place of phosphatase in bone formation It has been suggested that it has no direct role in calcification but is associated with formation of organic matrix This view finds strong support in the recent demonstration that calcification of cartilage reconstituted from solution will occur spontaneously at physiologic concentrations of calcium and phosphorus [25] The only requirement is that the cartilage (whatever the source) have the structure of ' native cartilage This poses the question as to why all native cartilage does not calcify It has been suggested that traces of an essential mucopolysaccharide [26] are present even in these purified solutions It has been shown that beryllium which inhibits alkaline phosphatase activity inhibits calcification This does not establish a role for phos-

phatase in the calcification process however since it has been shown that beryllium has this property in concentrations which do not affect liberation of inorganic phosphate from β glycerophosphate [27]

Finally it has been suggested that phosphatase serves to destroy phosphate esters which *inhibit* calcification as crystal poisons [28] In this view the phosphate esters which are promoting calcification in slice experiments protect phosphate-containing sites from phosphatase leached from the slices [21]

## BIOCHEMICAL DEFECTS IN HYPOPHOSPHATASIA

Although rachitic cartilage (and probably the uncalcified osteoid in osteomalacia) normally calcifies readily in normal concentrations of calcium and phosphate ions this does not occur in patients with hypophosphatasia In this syndrome the serum phosphorus level is normal and the serum calcium level is frequently elevated despite the presence of histologic rickets Furthermore Fraser showed directly that rachitic cartilage would calcify in the serum of a patient with hypophosphatasia This makes it unlikely that the defect in calcification results from too low an activity product of circulating calcium and phosphate ions The possibility does exist however that calcium may be present in a complexed nonionized form This is suggested by the observation that vitamin D in average doses may rapidly elevate the serum calcium concentration of patients with hypophosphatasia [9] Anning and associates [29] showed that vitamin D can increase significantly the concentration of diffusible nonionized calcium On the other hand if calcification depends upon the activity of phosphate locally at osteoid sites and this is dependent upon phosphatase the disorder may indeed involve a decrease of effective ion product

The syndrome thus provides strong clinical evidence that alkaline phosphatase plays a role in the calcification process Since osteoid is present in abundance it appears likely that phosphatase is not required for its formation There remains the possibility that osteoid formed without alkaline phosphatase is abnormal e g lacks a phosphorus containing compound required for in vivo calcification such as the mucopolysaccharide described by Distefano and associates [30] As noted above the rachitic cartilage of a patient with hypophosphatasia did not calcify in normal serum [12] Attempts to define further the relationship between the enzyme deficiency and the bone disease have proved unsuccessful

An inhibitor of alkaline phosphatase could not be found in the serum of patients although carefully sought for by four groups of investigators [9 10 16 20]

point is that calcification in vivo normally occurs despite a product of activities of $Ca^{++} \times HPO_4^{--}$ in serum below that required for spontaneous precipitation (Hydroxyapatite the mineral phase of formed bone will remove calcium and phosphate from solutions with activity product $Ca^{++} \times HPO_4^{--}$ below that of serum The present problem however, concerns a failure of calcification *de novo* of newly formed osteoid or calcifiable cartilage )

A number of attempts have been made to explain this paradox The first attempt (which antedated indeed, accurate knowledge of the appropriate activity products) was that of Robison [22] who noted the presence of alkaline phosphatase in all areas where calcification occurs He suggested that phosphatase acts to liberate inorganic phosphate at the site of calcification and thus, by raising the activity product of calcium by phosphate ions locally to initiate crystallization This view was strengthened by the observation that calcification of rachitic cartilage would not occur after inactivation of phosphatase save at concentrations of calcium and phosphate ions far above the physiologic range [23] The concept was rendered untenable, however by the demonstration that inhibitors of glycolysis not affecting alkaline phosphatase could inhibit calcification of cartilage in concentrations of calcium and phosphate well above the physiologic range It was shown that calcification is blocked by phlorizin iodoacetate or fluoride but that it can be restored in the presence of the first two if glucose 1 phosphate or 3 phosphoglycerate respectively is added these compounds appear "below" the block in glycolysis ATP itself would not promote calcification so it does not appear that the glycolytic cycle serves solely to supply energy [24]

Further this inhibition can be overcome by the addition of substrates (such as phenylphosphate) not in the pathway of glycolysis but readily hydrolyzed by phosphatase These observations led Gutman and Yu to propose that phosphatase acts as a transferase of phosphate from organic phosphate esters to available sites on the matrix [24]

Two other theories have been proposed to explain the place of phosphatase in bone formation It has been suggested that it has no direct role in calcification but is associated with formation of organic matrix This view finds strong support in the recent demonstration that calcification of cartilage reconstituted from solution will occur spontaneously at physiologic concentrations of calcium and phosphorus [25] The only requirement is that the cartilage (whatever the source) have the structure of native cartilage This poses the question as to why all native cartilage does not calcify It has been suggested that traces of an essential mucopolysaccharide [26] are present even in these purified solutions It has been shown that beryllium which inhibits alkaline phosphatase activity inhibits calcification This does not establish a role for phos

## THERAPY

There is no generally accepted therapy for hypophosphatasia Vitamin D has been tried (because the bone disease is the same as that found in rickets) with apparent improvement in one case [17] This same case and several other cases so treated [2 9 56] developed hypercalcemia and signs of vitamin D intoxication There is no further evidence that vitamin D deficiency plays a part in the disease or that therapy with vitamin D is effective

In a single case cortisone has been reported to produce rapid improvement in the radiologic defects in the bones and to raise the serum alkaline phosphatase concentration to twice pretreatment values although not to normal levels Relapse occurred when cortisone was omitted and there was a comparable response when it was reinstituted On the other hand four cases are said to have shown no response to cortisone [18 37-39]

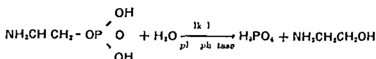
There is no record of the use of phosphate supplements as a therapeutic measure to induce hyperphosphatemia As noted above it is possible that acalcification in hypophosphatasia results from a subnormal ion product at osteoid sites normally overcome through an action of phosphatase It is possible that the defect could be overcome by inducing an abnormally high solubility product in circulating fluids

## GENETIC ASPECTS

Figure 43-3 shows the family tree given by McCance and associates [16] who studied two siblings who died in infancy It is of interest that low serum alkaline phosphatase values were found in two generations There was strong circumstantial evidence for the occurrence of hypophosphatasia in the maternal sibship of the father of the propositi

An analysis of the genetic aspects of hypophosphatasia by Steinberg is quoted in a recent review [3 ] He estimates that the incidence of the disease among siblings of propositi is 28 per cent notes no evidence of sex linkage and finds significantly more affected females than males From data based on serum phosphatase values alone he makes the following analysis The serum phosphatase level is low in 70 per cent of affected parents for whom data are available Assuming that the disease is transmitted as an autosomal recessive trait (and that all parents of patients are thus heterozygotic for hypophosphatasia) two-thirds of all clinically normal siblings of patients should be heterozygotic and about 50 per cent (0 70 × 0 66) should then have low serum phosphatase values This compares well with an observed ratio of three affected in seven clinically healthy siblings tested Steinberg further noted a high frequency of the disease in the last born sibling and concludes that it is

Phosphoethanolamine has been isolated from urine and plasma of patients and of some parents of patients Although alkaline phosphatase readily hydrolyzes this ester, there is no reasonable explanation for the association of the two biochemical defects in the disease It has been suggested that phosphoethanolamine is the true substrate for bone alkaline phosphatase [19] Thus, a hereditary block in the reaction




would lead to an excess of plasma and urinary phosphoethanolamine A block in hydrolysis of the ester could lead to a defect in calcification whether the phosphatase acts as a liberator of inorganic phosphate as a transferase or indeed, as a metabolic "poison" as suggested by Neuman [28] There is no evidence in favor of or against such a hypothesis Phosphoethanolamine is not normally found in serum or urine It is a normal constituent of brain tissue It may appear with amino acids in the urine of patients with liver disease [31], celiac disease and erythroblastosis fetalis [32]

Hypercalcemia and hypercalcinuria occur frequently but not constantly in hypophosphatasia When they do occur there may be nitrogen retention and nephrocalcinosis A serum calcium concentration greater than 11 mg per 100 ml was noted on one or more occasions in 20 of the 31 cases for whom data were available [2]

It has been observed [1] that the serum calcium may undergo wide fluctuations without apparent cause and [2] that hypercalcemia may result from therapy with moderate doses of vitamin D It may also occur in subjects who have never received vitamin D [3] Thus one patient developed a serum calcium concentration of 17 mg per 100 ml after 10 days on 50 000 units a day [9], another who had a serum calcium concentration of 12 to 15 mg per 100 ml before therapy with the vitamin showed a return of serum calcium level to normal while the patient was receiving 50 000 units a day [17]

Inasmuch as the bones are surrounded with osteoid and little or no osteoclastic activity is seen histologically it has been generally assumed that the hypercalcemia results from hyperabsorption of calcium Even with normal calcium absorption hypercalcemia might indeed develop if the bone matrix were unable to deposit calcium (as is apparently the case in hypophosphatasia) The occurrence of all the other abnormalities of hypophosphatasia in many patients with normal serum calcium who have not had vitamin D throughout their clinical course eliminates the possibility that the alkaline phosphatase in this disease is depressed as a result of hypervitaminosis D and hypercalcemia [33 34]

activity is decreased and phosphoethanolamine is found in plasma and urine There may be premature synostosis of the cranial vault, hypercalcemia nephrocalcinosis and premature loss of teeth

2 The disease may be detected first in infancy or in adulthood Diagnosis is based on the demonstration of radiologic and histologic changes in bone indistinguishable from those of rickets phosphoethanolamine in the urine and decreased serum alkaline phosphatase activity

3 The cardinal pathologic feature is inadequate calcification of bone matrix The biochemical basis for this and its relation to the other biochemical defects are not known It is possible that alkaline phosphatase is necessary for the formation of normal osteoid or that phosphoethanolamine normally has an integral place in the calcification process

4 The disease picture offers strong support for a role for alkaline phosphatase in normal calcification of bone

5 There is no adequate therapy

6 Available data suggest that the disease is inherited as an autosomal recessive trait Heterozygotes may have low serum alkaline phosphatase concentration high urinary excretion of phosphoethanolamine or both without bone disease

## BIBLIOGRAPHY


1 Rathbun J C Hypophosphatasia a new developmental anomaly Am J Dis Child 75 832 1948
2 Fraser D Hypophosphatasia Am J Med 22 730 1957
3 Currarino G Neuhauser L Reyersback C and Sobel E Hypophosphatasia Am J Roentgenol Radium Therapy Nuclear Med 78 392 1957
4 Huhne T and Schonfeld L Eine eigenartige Wachstumstorung im Kindesalter Monatsschr Kinderh 42 267 1929
5 Chown B Renal rickets and dwarfism a pituitary disease Brit J Surg 23 552 1935 1936
6 Kubatsch H Über eine seltene Knochenerkrankung Monatsschr Kinderh 75 253 1938
7 Arspach W E and Clifton W M Hyperparathyroidism in children Am J Dis Child 58 540 1939
8 Macey H B Multiple pseudofractures report of case Proc Staff Meet Mayo Clin 15 789 1940
9 Sobel E H Clark L C Fox R P and Robinow M Rickets deficiency of alkaline phosphatase activity and premature loss of teeth in childhood Pediatrics 11 309 1953
10 Fraser D Yendt E R and Christie F H Metabolic abnormalities in hypophosphatasia Lancet 1 286 1955
11 McCance R A The excretion of phosphoethanolamine and hypophosphatasia Lancet 1 131 1955
12 Fraser D and Yendt E R Metabolic abnormalities in hypophosphatasia A M A J Dis Child 90 552 1955
13 Schlesinger B E and Luder J F Further observations on hypophosphatasia A M A J Dis Child 92 433 1956
14 Rathbun J C Schlesinger N Stokes M and Bauer C Hereditary enzymatic effects as illustrated by hypophosphatasia Ann New York Acad Sc 75 2 9 1958
15 Park E A and Powers G F Acrocephaly and scaphocephaly with sym

possible that heredity may not be involved in the disease It may be of course that families with an affected child avoid future pregnancies or that medical reports appear before the families are complete

The analysis on which Steinberg s study was based does not include more recent data on phosphoethanolamine excretion among ancestors and siblings of patients When this valuable adjunct to the detection of heterozygosity is incorporated into a larger genetic study the hereditary

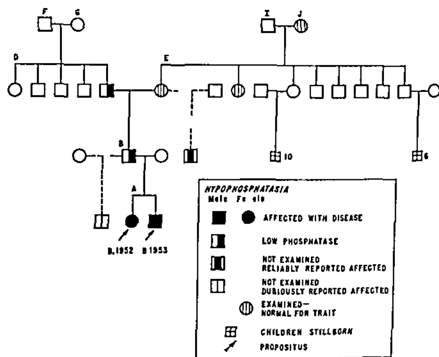



Fig 44-3 Pedigree of two infants with hypophosphatasia (*By permission of R A McCance et al [16]*)

aspects of hypophosphatasia should be considerably clarified [2] Fraser reported that this test brought the incidence of detectable heterozygosity *in parents of patients to 81 per cent He notes that there is no good* evidence that the defect of phosphatase and that of phosphoethanolamine metabolism are carried on the same gene and that there is suggestive evidence that they are not Relatives who excrete phosphoethanolamine tend to have higher serum phosphatase values than those who do not

## SUMMARY

1 Hypophosphatasia is a familial disease in which severe skeletal defects result from a failure of deposition of apatite in osteoid and of normal ossification at epiphyseal plates The serum alkaline phosphatase

# Chapter 45

# Hereditary Hypoproteinemias and Other Plasma Protein Abnormalities

*Robert S Gordon Jr*

During the past decade the availability of isotopic tracers and the application of the concept of the dynamic state of body constituents [1] together with increasingly effective procedures for the determination and isolation of specific proteins [2] have led to significant advances in the understanding of the physiology and biochemistry of the plasma proteins At the same time improved clinical chemical methods for the estimation of proteins and the great simplification of electrophoresis by the introduction of supporting media have stimulated the application of this new knowledge to the study of disease [3] It is not surprising therefore that this decade has been the period of discovery of disorders characterized by abnormal metabolism of plasma proteins Some of the important defects in plasma protein metabolism will be discussed in this chapter In these conditions deficiency or qualitative abnormality of the plasma protein is demonstrable by physical methods

Before the disorders of plasma protein metabolism are introduced it will be desirable to outline the normal metabolism of these constituents No plasma protein has been so extensively studied with regard to origin turnover and metabolic fate as albumin Its natural abundance relatively high stability the fact that it may be purified by crystallization and the availability of large quantities for intravenous administration to man make it particularly suitable for metabolic investigations For these reasons its metabolism will be discussed as an example of plasma protein turnover Far less is known of the turnover of the other plasma proteins although it seems reasonable to assume that their metabolism is similar to that of albumin with different rates of formation and destruction or utilization Gamma globulin has been shown to differ from albumin in one important respect it originates in peripheral tissues [4] probably in

metrically distributed malformation of the extremities Am J Dis Child 20 235 1920
15a Bauer W and Bartter F C Oxycephaly in *Textbook of Medicine* 10th ed edited by R L Cecil and R F Loeb p 1406 Saunders Philadelphia 1959
16 McCance R A Fairweather D V I Barrett A M and Morrison A B *Genetic clinical* biochemical and pathological features of hypophosphatasia Quart J Med N S 25 523 1956
17 Schlesinger B Luder J Bodian M Rickets with alkaline phosphatase deficiency an osteoblastic dysplasia Arch Dis Childhood 30 265 1955
18 Henneman P Personal communication
19 Dent C E *CIBA Foundation Symposium Bone Structure and Metabolism* p 266 Churchill London 1956
20 Engfeldt B and Zetterstrom R Osteodysmetamorphosis fetalis clinical pathological study of congenital skeletal disease with retarded growth hypophosphatasemia and renal damage J Pediatrics 45 125 1954
21 Neuman W F and Neuman M W *The Chemical Dynamics of Bone Mineral* University of Chicago Press Chicago 1958
22 Robison R Possible significance of hexosephosphoric esters in ossification Biochem J 17 286 1923
23 Waldman J Calcification of hypertrophic epiphyseal cartilage *in vitro* following inactivation of phosphatase and other enzymes Proc Soc Exper Biol & Med 69 262 1948
24 Gutman A B and Yu T F Concept of role of enzymes in endochondral calcification Josiah Macy Jr Foundation Tr Conf Metabolic Interrelations 2 167 1950
25 Glimcher M J Hodge A J and Schmitt F O Macromolecular aggregation states in relation to mineralization the collagen hydroxyapatite system as studied *in vitro* Proc Nat Acad Sc U S 43 860 1957
26 Kendrew J C in *The Proteins* edited by H Neurath and K Bailey vol II part B chap 23 Academic Press Inc New York 1954
27 Hiatt H H and Shorr E Inhibition of endochondral calcification *in vitro* by beryllium and L-histidine Josiah Macy Jr Foundation Tr Conf Metabolic Interrelations 3 105 1951
28 Neuman W F DiStefano V and Mulryan B J Surface chemistry of bone III Observations on role of phosphatase J Biol Chem 193 227 1951
29 Anning S T Dawson J Dolby D E and Ingram J T The toxic effects of calciferol Quart J Med 27 203 1948
30 DiStefano V Neuman W F and Rouser G The isolation of a phosphate ester from calcifiable cartilage Arch Biochem & Biophys 47 218 1953
31 Walshe J M Disturbances of amino acid metabolism following liver injury A study by means of paper chromatography Quart J Med N S 22 483 1953
32 Fisher O D and Neill D W Excretion of ethanolamine phosphoric acid in coeliac disease Lancet 1 334 1955
33 Nordin B E C and Fraser R *CIBA Foundation Symposium Bone Structure and Metabolism* p 222 Churchill London 1956
34 von Fanconi G and de Chastonay E Die D Hypervitaminose im Säugling salter Helvet paediat acta 5 5 1950
35 Steinberg A G quoted in Hypophosphatasia by G Currarino et al Am J Roentgenol Radium Therapy Nuclear Med 78 392 1957
36 Felder J and Schreirer K Hypophosphatasie Monatsschr Kinderh 103 437 1955
37 Laxdal O E quoted in Hypophosphatasia by D Fraser Am J Med 22 730 1957
38 Schlesinger B quoted in Hypophosphatasia by D Fraser Am J Med 22 730 1957
39 Dent C E quoted in Hypophosphatasia by D Fraser Am J Med 22 730 1957

with an amino acid sequence that appears to deviate from the normal only with regard to a small number of residues but in which the deviant synthesis is itself specific and is passed on unchanged from generation to generation

*Distribution and Catabolism of Albumin*

The distribution of serum albumin in man has been shown [16 17] to involve a volume considerably greater than that of the circulating plasma. The latter contains only about 40 per cent of the total exchangeable albumin. The presence of plasma proteins in lymph was emphasized many years ago by Drinker and Field [18] and the existence of immunologically identifiable plasma proteins in the interstitial fluid of muscle has been shown by Gitlin Landing and Whipple [19]. Albumin degradation appears to be a first-order process and a newly synthesized molecule is just as likely to be degraded as an old one. A semilogarithmic plot of the disappearance of labeled albumin yields a straight line, and the survival of the protein is measured by a half time

A recent study of the degradation of $I^{131}$ labeled albumin in man [20] indicates that the site of the metabolism of albumin is an extravascular compartment which is in rapid equilibrium with the circulating albumin. Animal experiments [21] suggest that the liver plays an important role in the catabolism as well as in the synthesis of albumin in vivo and studies *in vitro with surviving perfused liver [22] and with slices [23] demonstrate* hepatic albumin degradation. The further fate of compounds released by the degradation of albumin in vivo is not known although it has been shown in the intact animal that labeled amino acids from albumin eventually make their way into new proteins [24 25]. Albright et al [26] and Waterhouse Bassett and Holler [27] suggest that albumin contributes to the formation of new protoplasm in human subjects. In balance studies on subjects receiving albumin by vein they find retention of phosphorus which so far exceeds the retention of calcium that it cannot all be accounted for by bone formation

*Clinical Investigation of Albumin Metabolism*

Luck [28] has described in sobering detail the problems confronting the clinical investigator of *serum protein turnover*. *An isotopically labeled* protein is introduced into the circulation of the subject to be investigated and its rate of disappearance is followed. The performance of such a study is complicated from the start by the difficulties inherent in the isolation of a pure protein for administration and for isotopic assay. Other difficulties are *inherent in the* biologic behavior of the labeled protein. If it is tagged with $N^{15}$ $C^{14}$ or $S^{35}$ the commonly used isotopes of the elements

plasma cells [5] In addition, most of the plasma proteins cannot be isolated as single components for investigation The heterogeneity of γ globulin as usually defined seems to be reflected in a range of half times in survival studies [6]

## METABOLISM OF SERUM ALBUMIN

### *Chemistry of Albumin*

Serum albumin in crystalline form has been available for chemical investigation since the work of Cohn Hughes and Weare [7] in 1947 The studies that have been performed on this protein have made it by far the best known of the plasma proteins although its detailed structure has not yet been defined It probably consists of a single peptide chain, internally cross linked by disulfide bonds between cysteine residues [8] The terminal amino acids have been tentatively identified for human serum albumin the N terminal residue is aspartic acid [9 10], followed by alanine The C-terminal acid is leucine, with (albumin) glycine valine alanine leucine being the probable sequence of preceding residues [11] A discussion of the many physical characteristics and chemical properties of serum albumin that have been reported is beyond the scope of this chapter and is not essential for the present consideration of disturbances in its metabolism Edsall and Wyman [12] have incorporated the significant features of the physical chemistry of serum albumin into their recent monograph

### *Synthesis of Albumin*

Beyond the observation that incorporation of $C^{14}$ lysine into serum albumin occurs exclusively in the liver of the rat [13] virtually nothing is known of the details of its synthesis The processes of protein synthesis and their genetic control have been intensively investigated in systems simpler than mammalian liver this field has been reviewed recently by Anfinsen [14]

The question of the specificity of protein synthesis and structure is of the greatest importance in a discussion of the disorders of serum protein metabolism In a current review of this field Vaughan and Steinberg [15] advance the belief that all the proteins that have been adequately characterized are synthesized by mechanisms of high specificity so that each molecule of the protein under consideration is identical with each other at least as regards amino acid sequence This tenet is supported by the apparent homogeneity throughout a wide variety of studies of human and bovine serum albumins even when the proteins are prepared from pooled serums representing many individuals This concept of specificity of chemical structure of the protein renders intelligible the investigations of the hereditary variation discussed below in which an albumin occurs

## ABNORMALITIES OF SERUM ALBUMIN METABOLISM

### DOUBLE ALBUMIN ANOMALY (BISALBUMINEMIA)

This condition which cannot properly be called a disease has been described in at least six kindreds within the past 5 years Since the abnormality can be detected only by electrophoretic analysis of the plasma proteins and since it appears to produce no untoward effects in its bearers it is hardly surprising that it had not come to medical attention earlier As an inherited trait which can be measured quantitatively it is of interest to the geneticist and familiarity with the condition may prevent unnecessary excitement on the part of physicians who encounter the anomaly in the future It seems appropriate to mention it in connection with other disorders of plasma protein metabolism

The first mention of the anomaly was made by Scheurlen [35] who noticed a double albumin peak in the electrophoretic diagram of the plasma proteins of one of a series of diabetic patients This case may actually not have been typical since the separation of the two peaks in the electrophoretic diagram appears to have varied with changes in the clinical condition of the patient In a later report [36] it is stated that the same anomaly was encountered in two relatives of this original patient and the anomaly is equated by the authors with more clear-cut examples of the disorder Subsequent reports by Knedel [37 38] Nennstiel and Becht [39] Earle Hutt Schmid and Gitlin [40 41] and by Bennhold Ott and Scheurlen [36] have brought to six the number of kindreds in which the condition is recognized The family studied by Earle et al is the largest containing 25 proved and 3 presumed cases These authors have carried out studies of the pattern of heredity and of the chemistry of the abnormal protein The discussion which follows is based primarily on their observations

#### *Biochemical Features of Albumin B*

The anomaly consists of a double albumin peak in the electrophoretic pattern of serum The combined areas are equal to the area of a normal albumin component The faster moving component has the mobility of normal albumin and cannot be differentiated from it the slower moving component (called albumin B by Earle et al ) is superimposed on the $\alpha_1$ globulin The two albumins do not separate in the ultracentrifuge are both found in Cohn fraction V and become equal in electrophoretic mobility at pH values above 11 3 and below 3 5 The quantitative precipitin reaction with rabbit antiserum and the Ouchterlony agar diffusion technique do not differentiate albumin B from normal human serum albumin These observations may be explained satisfactorily by the hypothesis that albumin B is in fact identical with normal serum albumin

naturally occurring in proteins the investigator must estimate the extent to which these isotopes liberated by the breakdown of body or plasma protein may be reutilized in the synthesis of new protein If, on the other hand, it bears $I^{131}$ the most commonly employed extraneous isotopic label there is the ever present possibility that the labeled molecules may be differentiated metabolically from the natural ones These considerations make it impossible to obtain an absolutely precise measurement of the rate of albumin turnover in man but they detract only slightly from the usefulness of labeled proteins in the study of clinical problems associated with disturbed albumin metabolism By applying the same procedure to subjects under study and to normal controls one can at least make a valid estimate of the direction and extent of the differences encountered

In the procedure of Armstrong et al [29 30], the patients label their own proteins after ingestion of isotopic precursors contained in yeast grown in the presence of $S^{35}$ The rates of incorporation and loss of isotope from the circulating plasma proteins can then be followed It is apparent that this technique does not permit the calculation of total body albumin content by isotope dilution A further disadvantage stems from the labeling of all body proteins and in fact all sulfur compounds undergoing active metabolism so that there is a very considerable pool of isotope available for reincorporation into plasma proteins as it is released For this reason, the absolute rates of catabolism of albumin estimated by this procedure are erroneously low, but the procedure is a simple one, and probably suitable for the comparison of patients with control subjects Many of the objections that might be raised to the above mentioned procedure were avoided by Volwiler et al [31], who administered isotopic methionine to one subject and then transferred labeled plasma proteins from this subject to others whose turnover rate was to be investigated Unfortunately this procedure is too cumbersome for widespread clinical use and involves hazards (of radiation for the plasma donor and hepatitis for the recipient) that sharply limit its applicability

In contrast to the techniques just described which use isotopes of atoms naturally occurring in proteins the most popular procedure [16 17 20] has been the study of albumin distribution and degradation using protein labeled with $I^{131}$ Cohen et al [32] and Campbell et al [33] have presented evidence that $I^{131}$ labeled protein behaves very much like $C^{14}$ protein in experimental animals and a recent study by Freeman et al [34] demonstrates that $I^{131}$ albumin and native albumin are degraded at the same rate in a patient with analbuminemia The easy availability of $I^{131}$ albumin the simplicity and accuracy of its assay and the advantages of the isotope in minimizing radiation hazard to the patient have made this compound a favorite tool for clinical research

kindreds 13 of 18 have the anomaly When one remembers that there are necessarily 6 affected propositi it may be seen that the probability of inheriting the trait from an affected parent is not significantly different from 0.5 The e data are interpreted by Earle et al as consistent with the hypothesis that there are two genetic loci controlling the synthesis of albumin that these loci are independent and that in the heterozygote *there is equal production of normal albumin and albumin B* These authors consider that the anomaly is transmitted as a codominant characteristic both the abnormal and the normal genes being fully expressed The homozygotic abnormal situation has not been encountered to date Whether or not such a homozygotic individual would suffer from some clinically detectable disease cannot be predicted on the basis of presently available information

## ANALBUMINEMIA

### *Historical Aspects*

Bennhold Peters and Roth [42] first described a pair of siblings from whose plasma albumin was absent Subsequent communications from the clinic at Tübingen [43 44] have amplified the original description and presented the results of a variety of biochemical investigations Recently three more analbuminemic subjects have been reported two in the United States and one in Switzerland [45–47] The former two patients have been under observation since 1952 and 1956 respectively the latter *was only recently discovered Pending the identification of* more individuals with this disorder the description of the disease must be based on this small series and generalizations must be regarded as tentative

### *Clinical Features*

The most striking feature of analbuminemia is the relative paucity *of symptoms and signs* Edema *usually of mild degree* has been noted in all cases but one and has been relieved by the administration of albumin in the two individuals who received full replacement doses The significance of edema cannot be assessed in the patient reported by Shetlar et al [47] because of the coincident occurrence of congestive heart failure Two subjects [44 45] have received replacement therapy with large amounts of exogenous human serum albumin and have noted an increased sense of well being which may be taken as evidence of a preexisting mild malaise The two young male patients however feel well enough to carry on heavy manual labor [43 46] Bennhold and his colleagues [42] observed that their two patients with analbuminemia had a moderate degree of arterial hypotension a feature which they considered to be an adaptation which helps to prevent excessive edema for

except that two or more cysteine or tyrosine residues replace residues of dicarboxylic acids in the structure of the molecule

Individuals who have the albumin B anomaly quite uniformly have approximately equal quantities of normal albumin and of albumin B Earle et al state that albumin B constitutes 57 ± 4 4 per cent of the total albumin The failure of electrophoresis to separate albumin B from $\alpha_1$ globulin necessarily introduces a systematic error into this estimate

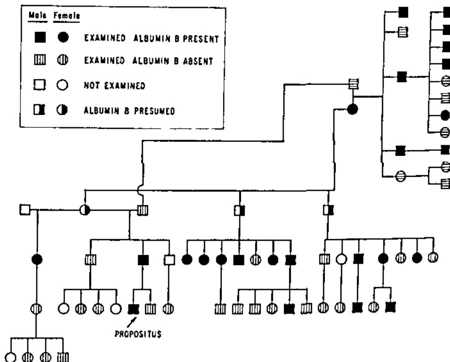



Fig 45-1 Genealogy of double albumin anomaly *(Redrawn after Earle et al [40])*

The authors feel that a correction for the average amount of $\alpha_1$ globulin would make the quantities of the two albumins virtually identical in all their cases As yet no study of the relative turnover rates of the two albumins has been effected

## *Genetics*

The pattern of heredity may be fairly clearly defined from the accounts of double albumin anomaly which have been published The genealogy of the family studied by Earle et al is reproduced in Fig 45 1 Since there is no known case in which the anomaly was found in the offspring of unaffected parents it may be presumed that only the offspring of an affected parent are exposed to genetic risk In the family studied by Earle et al 28 of 46 individuals at risk are affected in the other 5

kindreds 13 of 18 have the anomaly When one remembers that there are necessarily 6 affected propositi it may be seen that the probability of inheriting the trait from an affected parent is not significantly different from 0.5 These data are interpreted by Earle et al as consistent with the hypothesis that there are two genetic loci controlling the synthesis of albumin that these loci are independent and that in the heterozygote there is equal production of normal albumin and albumin B These authors consider that the anomaly is transmitted as a codominant characteristic both the abnormal and the normal genes being fully expressed The homozygotic abnormal situation has not been encountered to date Whether or not such a homozygotic individual would suffer from some clinically detectable disease cannot be predicted on the basis of presently available information

### ANALBUMINEMIA

#### *Historical Aspects*

Bennhold Peters and Roth [42] first described a pair of siblings from whose plasma albumin was absent Subsequent communications from the clinic at Tubingen [43 44] have amplified the original description and presented the results of a variety of biochemical investigations Recently three more analbuminemic subjects have been reported two in the United States and one in Switzerland [45–47] The former two patients have been under observation since 1952 and 1956 respectively the latter was only recently discovered Pending the identification of more individuals with this disorder the description of the disease must be based on this small series and generalizations must be regarded as tentative

#### *Clinical Features*

The most striking feature of analbuminemia is the relative paucity of symptoms and signs Edema usually of mild degree has been noted in all cases but one and has been relieved by the administration of albumin in the two individuals who received full replacement doses The significance of edema cannot be assessed in the patient reported by Shetlar et al [47] because of the coincident occurrence of congestive heart failure Two subjects [44 45] have received replacement therapy with large amounts of exogenous human serum albumin and have noted an increased sense of well being which may be taken as evidence of a preexisting mild malaise The two young male patients however feel well enough to carry on heavy manual labor [43 46] Bennhold and his colleagues [42] observed that their two patients with analbuminemia had a moderate degree of arterial hypotension a feature which they considered to be an adaptation which helps to prevent excessive edema for

mation Of the three patients reported recently, one has normal blood pressure [50] one has a possibly slightly reduced value [46] and one [47] having aortic insufficiency cannot be considered in this respect

Associated disorders or abnormalities are present in several of these analbuminemic subjects, but these features each existing in only one case, cannot be considered an integral part of the picture of albumin deficiency The patient reported by Shetlar et al [47] suffers from rheumatoid arthritis of severe degree and from aortic valvular insufficiency leading to congestive heart failure He had sought medical aid for these conditions, and the analbuminemia was an incidental discovery The patient studied by Bartter and collaborators [50] suffers from the deformities typical of progressive lipodystrophy and has required surgical treatment for varicose veins The subject seen by Beck and Dorta [46] gave a history of cranial injury in childhood and subsequent convulsive episodes, he also was noted to have atrophic testes and a mild degree of feminization He had developed a dermatitis from handling cement and had sought medical attention for that disorder

The generally good health enjoyed by these patients in spite of their analbuminemia makes it impossible to assign an age of onset to the condition In four of the five subjects the anomaly was documented during young adult life and three of the five had been followed for over 6 years with no essential alteration in the condition It therefore seems likely that analbuminemia has been present since birth although the possibility that it is an acquired defect cannot be eliminated

*Biochemical Aspects*

The term *analbuminemia* implies complete absence of serum albumin from the blood of these individuals The available evidence suggests that traces of albumin may be found by suitably exact methods but the quantities are so small that it seems justifiable to retain the present nomenclature The most thoroughly investigated subjects, from this point of view, are those at Tübingen In each of these, electrophoretic examination of serum revealed no albumin One subject was then treated with exogenous albumin the persistence of which makes other, more refined investigations impossible The other patient however, received no replacement therapy and has been reinvestigated more recently Lohss and Kallee [48] report the use of an elaborate procedure involving radioiodination immunologic precipitation and electrophoresis with the conclusion that if albumin is present the concentration must be less than 10 mg per 100 ml Later however Lohss and Ott [49] succeeded in demonstrating the presence of traces of albumin by immunoelectrophoresis Virtually identical findings are reported in the case of Beck and Dorta [46] although no albumin peak can be discerned on electrophoretic examination of whole serum immunoelectrophoresis in the

presence of an antialbumin serum reveals the presence of a trace of albumin The case reported by Shetlar et al [47] was found to have less than 0 1 gm albumin per 100 ml serum with good agreement between results by electrophoretic and immunochemical methods The case reported from the National Institutes of Health [49] had unfortunately been treated with exogenous albumin before any accurate measurements of serum albumin concentration were made

The deficiency of serum albumin in analbuminemia can be attributed to failure of albumin production not to an exaggerated rate of removal of albumin from the plasma The disappearance of exogenous albumin administered to these subjects is indeed abnormally slow in the three instances in which it has been studied [44 47 50] In two instances the rate of removal of $I^{131}$ labeled albumin has also been shown to be delayed [44 49] The failure of albumin production is not associated with other evidence of impaired liver function The other plasma protein fractions are not decreased and in two cases [45 47] liver biopsy has been carried out without the discovery of significant morphologic abnormalities Beck and Dorta [46] performed a liver biopsy on their patient and assayed the tissue for a variety of enzymes They noted a marked reduction in the activities of lactic malic and isocitric dehydrogenases and also decreased activity of glutamic-oxalacetic transaminase The variability of their normal values for transaminase however is so great that this last finding is not clearly abnormal They do not comment on morphology of the liver tissue

Certain other biochemical abnormalities observed in the patients with analbuminemia may be considered to be effects direct or indirect of the lack of albumin The concentration of serum globulins averages slightly above normal in the group although no one component is strikingly increased The results of laboratory procedures which depend upon serum protein concentrations (cephalin flocculation thymol turbidity Takata Ara test) may be abnormal The erythrocyte sedimentation rate is also uniformly elevated Serum calcium concentration is reduced and there is a considerable increase in serum cholesterol which was found to be as high as 610 mg per 100 ml in one instance [46] and over 400 mg per 100 ml in another [50] In the two patients who have received adequate albumin replacement these abnormalities have been corrected

It is hardly possible to say that all the biochemical functions of serum albumin have been described As the chief contributor to the colloid osmotic pressure of the plasma albumin fulfills a role in the maintenance of physiologic relationships between intravascular and extravascular fluid volumes Its vehicular function suspected long ago by Bennhold [51] has been emphasized by recent studies of the binding of unesterified fatty acids by albumin [52] and the demonstration of the important role of this complex in the transport of fatty acids [53] The patients with

analbuminemia are of great interest in so far as they demonstrate conclusively that neither these, nor any functions of albumin yet to be discovered are essential for life

*Treatment*

Analbuminemia can be corrected in only one way—the infusion of sufficient human albumin to repair the defect (It is to be hoped that cases discovered in the future will be studied exhaustively before albumin

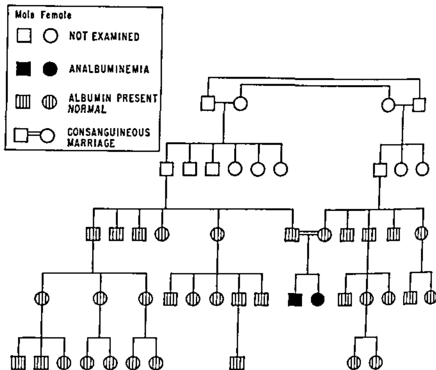



Fig 45-2 Genealogy of analbuminemia (*Redrawn after Bennhold [43]*) Symbols are the same as in Fig 45-1

supplementation is begun ) The quantity of albumin required to maintain a reasonably normal blood level is in the range of 25 gm per week because of the slow degradation in these persons This amount can safely be tolerated in one infusion unless some associated condition presents a contraindication The advisability of instituting albumin treatment is not established on the basis of existing knowledge The mildness of the symptoms the apparently good prognosis and the availability of alternative means for the control of edema may be taken as arguments against the recommendation of costly and time consuming albumin treatment On the other hand the patients so treated appear to feel better and their

hypercholesterolemia which might contribute to the development of premature atherosclerosis is thereby corrected

*Genetics*

A discussion of the pattern of inheritance of analbuminemia is not warranted at this time, since there are insufficient data to support any conclusion. There is no known example of transmission of analbuminemia from parent to offspring and the belief that it is a genetically determined anomaly is based solely on the existence of the condition in two siblings. The genealogy of these subjects has been published by Bennhold [43] and is reproduced in Fig 45-2. It may be seen that these patients are the offspring of a consanguineous marriage so that there is a good possibility of an autosomal recessive pattern of inheritance. Beck and Dorta [46] suggest that there may also be some inbreeding in the ancestry of their patient although consanguinity between his parents was not known to exist. No other cases of analbuminemia have been found among his relatives [46] nor among the available relatives of the two American patients [47 50]

## AGAMMAGLOBULINEMIA

*Historical Aspects*

This disorder which has received considerable attention over the past 8 years was first described by Bruton in 1952 [54]. The pertinent literature has been reviewed by Good and Zak [55] and by Gitlin Gross and Janeway [56] the latter presentation covers also the metabolism of this protein fraction and other diseases associated with abnormal serum concentrations. Agammaglobulinemia is customarily subdivided into congenital and acquired types. The former exists as the name implies from birth but is not manifest until passively transferred maternal $\gamma$ globulin has been metabolized. Acquired agammaglobulinemia may develop at any age and in either sex; the distinction between the two forms in an individual patient is frequently impossible

*Metabolic Defect*

The metabolic defect in agammaglobulinemia consists of failure of $\gamma$ globulin synthesis. The rate of degradation of $\gamma$ globulin has been studied both with isotopic tracers [57–59] and by following the loss from the plasma of therapeutic doses of passively infused $\gamma$ globulin [6 55 59] it is not increased. Failure of antibody synthesis is not complete however. In almost every case Gitlin et al [56] and Good and Zak [55] have been able to demonstrate small amounts of $\gamma$ globulin by immunochemical procedures. In four cases studied at the National Institutes of Health [6] the serum contained small amounts of electrophoretically demon

strable γ globulin For this reason the term *agammaglobulinemia* is a misnomer but it is sanctioned by common use

Agammaglobulinemia differs from analbuminemia in one important respect While the liver in analbuminemia is morphologically intact the tissues of agammaglobulinemic subjects show a great deficiency or total absence of plasma cells In addition, the lymph nodes are unusual in that they contain fewer lymphocytes than normal, and show ill-defined primary follicles [59, 60] One may therefore suspect that the primary lesion in agammaglobulinemia is not solely a failure of the synthesis of one specific protein but that in some way it prevents the formation or maturation of the cells which should produce the protein The condition might be considered closely allied to the familial structural malformations that have long been known to the anatomist

*Clinical Features*

The clinical manifestations of agammaglobulinemia are secondary to the failure of antibody synthesis and the resulting susceptibility to infection Gitlin et al [56] state that in their experience immunity to viral infections is not impaired but that the infections of agamma globulinemic children are of bacterial origin An interesting consequence of the lack of antibodies is that affected patients may not reject tissue homografts Good and Varco [61] demonstrated the acceptance of a skin homograft in an agammaglobulinemic boy and this principle was employed therapeutically by Martin et al [62] who transplanted lymph node tissue into an agammaglobulinemic adult Subsequently there was immunochemical evidence of some renewal of antibody synthesis Gitlin et al [63] have pointed out the surprising fact that agammaglobulinemic subjects frequently develop the manifestations of rheumatoid arthritis or less often other collagen diseases The significance of the occurrence of these conditions in patients who ought to be incapable of developing hypersensitivity is not yet known

*Diagnosis*

The diagnosis of agammaglobulinemia is established on the basis of chemical examination of the serum for γ globulin Electrophoresis and immunochemistry are the methods most commonly employed for the definitive test *The condition should be suspected in any patient* especially a male infant who manifests frequent bacterial infections and whose low resistance cannot readily be explained on any other basis Since many such patients have repeated respiratory infections they may develop chronic pulmonary disease before the underlying condition is recognized It is frequently possible to rule out agammaglobulinemia by the demonstration of normal blood type agglutinins If these are absent in any but a type AB subject further examination of the serum

is indicated Good and Zak [55] however note that an occasional agammaglobulinemic patient may have anti B agglutinin in his serum

*Therapy*

The accepted treatment for agammaglobulinemic patients consists of passive infusion of pooled normal human γ globulin combined with antibiotics to suppress infections Present knowledge does not permit the prescription of a standardized program of therapy, treatment must be individualized The antibiotics to be employed depend on the infecting organisms and their sensitivities γ globulin should be administered intramuscularly in sufficient dosage to maintain a reasonable plasma level No single therapeutically effective level can be established with certainty the proper dosage must be ascertained empirically for each patient A plasma γ globulin concentration of about 0 3 gm per cent is a reasonable initial goal its achievement in an average 70 kg subject having no endogenous γ globulin will require a priming dose on the order of 25 gm protein Maintenance of this level will require the administration of approximately 4 gm per week The poor prognosis of untreated agammaglobulinemia can be considerably improved by careful management

*Genetic Aspects*

The acquired form of agammaglobulinemia is a sporadically occurring disease *without known familial tendencies The congenital form on the*

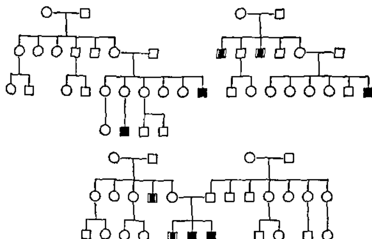


Fig 45-3 Genealogy of congenital agammaglobulinemia (*Drawn after Gitlin et al* [63]) *Solid square* = agammaglobulinemia *Part II, open square* = presumably affected—early death with history of recurrent severe bacterial infections *Open square* = presumably normal The authors do not specify how many of these subjects were examined professionally

other hand, appears to follow a definite genetic pattern Diagnostic problems make the study of this condition difficult, since the disease may not be differentiated simply from physiologic hypogammaglobulinemia of infancy, on the one hand or from acquired agammaglobulinemia on the other Nevertheless it is apparent that the congenital disease shows an overwhelming preponderance in the male sex. The few cases reported in female infants may be early examples of acquired agammaglobulinemia Gitlin Janeway Apt, and Craig [63] have diagrammed three kinships in which are found five proved and four presumed cases of agammaglobulinemia These genealogies are reproduced in Fig 45-3 In all instances the relationship between affected individuals is that of brothers or nephew and maternal uncle Occurrence of cases in brothers is noted by Good and Zak [33] and in cousins by Porter [64] The e observations give strong support to the belief that congenital agammaglobulinemia is inherited as a sex linked recessive characteristic

## OTHER INHERITED VARIATIONS IN PLASMA PROTEIN PATTERNS

Recent investigations utilizing the starch gel electrophoresis procedure described by Smithies [65] have brought to light several other variations in plasma protein patterns which appear to be inherited characteristics None of these anomalies appears to be associated with any disease Smithies and Walker [66] showed that normal human serums could be divided into three types on the basis of differences in the patterns obtained by their method of analysis It soon became clear that the protein fractions on which this differentiation was made were the previously described haptoglobins normal constituents of the serum which have the peculiar property of binding hemoglobin These haptoglobin types have proved to be hereditary characteristics but the precise genetic mechanism of their transmission is not clear An interesting hypothesis that might explain the findings is offered by Allison [67] Individuals who e serum contains no haptoglobin have also been encountered [68] but it has not yet been shown that the defect has a genetic basis

Several recent reports describe the findings of familial variants in the $\beta$ globulin components revealed by starch gel electrophore is Smithies [69] found evidence for the occurrence of three types of $\beta$ globulin and a recent communication by Harris Robson and Sini calco [70] increases the number to five All subjects so far reported have in their serum the $\beta$ globulin designated C and in most individuals this protein predominates In a number of Negroes and Australian aborigines however a slower moving protein designated D by Smithies [69] comprises about half the $\beta$ globulin In a few Caucasians a roughly equal amount of a faster moving component called B has been encountered That these

variants are hereditary is shown in genealogies given by Smithies [69] and by Harris et al [70]

Fraser Harris and Robson [71] have reported the presence in one family of a plasma protein not usually seen in human plasma The nature and significance of the component have not been elucidated but it appears not to have any detrimental effect on the members of the family who have it

Finally, mention should be made of the occurrence in one small family group of an abnormally great thyroxin binding capacity of the serum α globulin This anomaly recently reported by Beierwaltes and Robbins [72] seems to be of clinical significance only because it results in an unusually elevated protein bound iodine value in euthyroid subjects In one *individual discovered by Tanaka and Starr [73] an unusually low pro*tein bound iodine level seems to be related to the absence of thyroxin binding protein There is as yet no evidence for a familial tendency or genetic basis for this defect

## OTHER HYPOPROTEINEMIAS

Familial defects in plasma protein constituents are either responsible for or closely associated with several diseases discussed in other chapters of this book (Chaps 25 35 and 44) In addition there is the hitherto poorly defined entity of idiopathic hypoproteinemia Many such cases have been shown to have a defect allowing loss of macromolecules from the plasma into the gastrointestinal tract This defect can be conveniently demonstrated with $I^{131}$ labeled polyvinylpyrrolidone [74] Actual loss of *protein* into the lumen of the stomach has been well demonstrated in one case studied by Citrin Sterling and Halsted [75] The hypoproteinemia resulting from this protein losing gastroenteropathy should not at present be considered a biochemical lesion and no hereditary trend has yet been demonstrated

An unusual family several members of which had abnormal serum protein patterns by electrophoresis was described by Homburger and Petermann [76] It seems unlikely that the situation described is actually a hereditary biochemical anomaly since the manifestations were so varied and since *many of the cases had protein concentrations which* differed only slightly from the normal The more severely affected individuals may actually have had protein losing gastroenteropathy if so they will comprise the first family group known to be so affected

## SUMMARY

1 A number of variations in plasma protein patterns are known to be inherited characteristics Some involving the absence of a protein are

associated with disease Other conditions involving only a minor change in the chemistry of the protein while demonstrable by examination of the serum, do not produce any deleterious effects The important hereditary protein anomalies are as follows

2 Analbuminemia is an extremely rare condition characterized by failure of albumin synthesis The affected subjects have mild edema but are otherwise well There is virtually no albumin in the serum and laboratory tests that are affected by serum protein concentrations are abnormal Albumin replacement therapy is satisfactory and probably beneficial The mode of inheritance has not been established but the condition is probably transmitted by a recessive gene

3 Agammaglobulinemia is characterized by a marked reduction but usually not total absence of $\gamma$ globulin Since the subjects are unable to form antibodies, they are unusually susceptible to bacterial infection the consequences of which dominate the clinical picture Antibiotic and $\gamma$ globulin replacement therapy are helpful Agammaglobulinemic patients lack plasma cells the normal source of antibodies The clinical disease may be acquired or congenital The congenital form appears to be transmitted as a sex linked recessive characteristic

4 There are several conditions characterized by minor alterations in the chemistry of one or another plasma protein These variants are detected by electrophoresis on paper or starch gel None is associated with illness This category includes double albumin anomaly and the genetically controlled variants in $\beta$ globulins and haptoglobins

## BIBLIOGRAPHY


1 Schoenheimer R *The Dynamic State of Body Constituents* Harvard University Press Cambridge 1942
2 Cohn E J in *Blood Cells and Plasma Proteins Their State in Nature* edited by J L Tullis Academic Press Inc New York 1953
3 Durrum E L Laboratory aids to diagnosis and therapy (paper chromatography and electrophoresis) Ann Rev Med 9 451 1958
4. Miller L L Bly C G and Bale W F Plasma and tissue proteins produced by non hepatic rat organs as studied with lysine-ε-C[14] J Exper Med 99 133 1954
5 Ehrich W Cellular sources of antibodies in *Blood Cells and Plasma Proteins Their State in Nature* edited by J L Tullis Academic Press Inc New York 1953
6 Martin C M Gordon R S Felts W R and McCullough N B Distribution and metabolism of antibodies and gamma globulin in hypogammaglobulinemic patients J Lab & Clin Med 49 607 1957
7 Cohn E J Hughes W L Jr and Weare J H Preparation and properties of serum and plasma proteins XIII Crystallization of serum albumins from ethanol water mixtures J Am Chem Soc 69 1753 1947
8 Hunter M J and McDuffie F C Molecular weight studies on human serum albumin after reduction and alkylation of disulfide bonds J Am Chem Soc 81 1400 1959
9 Thompson E O P The N terminal sequence of serum albumins observations on the thiohydantoin method J Biol Chem 208 565 1954
10 Brown H Free amino groups of human serum albumin Science 121 106 1955

11 White W F, Shields, J and Robbins, K C C-terminal sequence of crystalline bovine and human serum albumins relationship of C-terminus to antigenic determinants of bovine serum albumin J Am Chem Soc 77 1267 195J
1 Edsall J T and Wyman J *Biophysical Chemistry* vol I *Thermodynamics Electrostatics and the Biological Significance of the Properties of Matter* Academic Press Inc New York 1958
13 Miller L L Bly C G Watson M L and Bale W F The dominant role of the liver in plasma protein synthesis J Exper Med 94 431 19J1
14 Anfinsen C B *The Molecular Basis of Evolution* Wiley New York 1J59
1J Vaughan M and Steinberg D The specificity of protein biosynthesis in *Advances in Protein Chemistry* vol 14 Academic Press Inc New York 1J59
16 Sterling K The turnover rate of serum albumin in man as measured by I[1] tagged albumin J Clin Invest 30 1228 1J51
17 Berson S A Yalow R S Schreiber S S and Post J Tracer experiments with I labeled human serum albumin distribution and degradation studies J Clin Invest 32 ı46 19J3
18 Drinker C K and Field M E The protein content of mammalian lymph and the relation of lymph to tissue fluid Am J Physiol 97 32 1J31
19 Gitlin D Landing B H and Whipple A The localization of homologous plasma proteins in the tissues of young human beings as demonstrated with fluorescent antibodies J Exper Med 97 163 19J3
20 Lewallen C G Berman M and Rall J E Studies of iodoalbumin metabolism I A mathematical approach to the kinetics J Clin Invest 38 66 1959
21 Gitlin D Klinenberg J R and Hughes W L Site of catabolism of serum albumin Nature London 181 1064 1958
' Cohen S and Gordon A H Catabolism of plasma albumin by the perfused rat liver Biochem J 70 544 19J8
23 Roberts S and Kelley M B Metabolism of plasma proteins in vitro J Biol Chem 222 55J 19J6
24 Yule C L Lamson B G Miller L L and Whipple G H Conversion of plasma protein to tissue protein without evidence of protein breakdown J Exper Med 93 J39 1951
5 Fleischer S Lietze A Walter H and Haurowitz F Conversion of serum proteins into tissue proteins Proc Soc Exper Biol & Med 101 860 1959
6 Albright F Forbes A P Bartter F C Reifenstein E C Bryant D Cox L D and Dempsey E F Studies on the fate of intravenously administered human plasma proteins in idiopathic hypoproteinemia and in osteoporosis in *Symposia on Nutrition* vol II *Plasma Proteins* The Robert Gould Research Foundation Inc Charles C Thomas Springfield Ill 1J50
27 Waterhouse C Bassett S H and Holler J W Metabolic studies on protein depleted patients receiving a large part of their nitrogen intake from human serum albumin administered intravenously J Clin Invest 28 ?4J 1J1J
28 Luck J M On the turnover of plasma proteins Am J Med 20 317 1J56
9 Armstrong S H Jr McLeod K Wolter J and Kukral J The persistence in the blood of the radioactive label of albumin gamma globulin globulins of intermediate mobility studied with S and paper electrophoresis method and preliminary results J Lab & Clin Med 43 918 1J54
J0 Armstrong S H Jr Bronsky D and Hershman J The persistence in the blood of the radioactive label of albumins gamma globulins and globulins of intermediate mobility III Comparison of die-away plots following oral and intravenous administration of the S label to the same subjects J Lab & Clin Med 46 857 1J55
31 Volwiler W Goldsworthy P D MacMartin M P Wood I A MacKay I R and Fremont-Smith K Biosynthetic determination with radioactive sulfur of turnover rates of various plasma proteins in normal and cirrhotic man J Clin Invest 34 11J6 1955
3? Cohen S Holloway R C Matthews C and McFarlane A S Distribution and elimination of I and C-labelled plasma proteins in the rabbit Biochem J 62 143 19J6

33 Campbell R M Cuthbertson D P Matthews C and McFarlane A S Behaviour of $^{14}C$- and $^{131}I$ labelled plasma proteins in the rat Internat J Applied Rad & Isotopes 1 66 1956
34 Freeman T Matthews C M L McFarlane A S Bennhold H and Kallee E Albumin labelled with iodine 131 in an analbuminaemic subject Nature London 183 606 1959
35 Scheurlen P G Über Serumeiweissveranderungen beim Diabetes mellitus Klin Wehnschr 33 198 1955
36 Bennhold H Ott H and Scheurlen G Beitrage zur Frage der genbedingten Bluteiweissstorungen Verhandl deutsch Gesellsch inn Med 64 2 J 1958
37 Knedel M Die Doppel Albuminamie eine neue erbliche Proteinanomalie Blut 3 129 1957
38 Knedel M Über eine neue vererbte Protein Anomalie Clin chim acta 3 72 1958
39 Nennstiel H J and Becht Th Über das erbliche Auftreten einer Albumin spaltung im Elektrophoresediagramm Klin Wehnschr 35 689 1957
40 Earle D P Hutt M P Schmid K and Gitlin D A unique human serum albumin transmitted genetically Tr A Am Physicians 71 69 1958
41 Earle D P Hutt M P Schmid K and Gitlin D Observations on double albumin a genetically transmitted serum protein anomaly J Clin Invest 38 1412 1959
42 Bennhold H Peters H and Roth L Über einen Fall von kompletter Anal buminaemie ohne wesentliche klinische Krankheitszeichen Verhandl deutsch Gesellsch inn Med 60 630 1954
43 Bennhold H Kongenitale Defektdysproteinamien Verhandl deutsch Gesellsch inn Med 62 657 1956
44 Bennhold H and Kallee L Comparative studies on the half life of $I^{131}$ labeled albumins and nonradioactive human serum albumin in a case of analbuminemia J Clin Invest 38 863 1959
45 Gordon R S Jr Bartter F C and Waldmann T Idiopathic hypoalbuminemias clinical staff conference at the National Institutes of Health Ann Int Med 51 553 1959
46 Beck G E and Dorta T Un cas d analbuminémie Helvet med acta 26 64 1959
47 Shetlar M R Payne R W Stidworthy G and Mock D Absence of serum albumin associated with rheumatoid arthritis Ann Int Med 51 1379 1959
48 Lohss F and Kallee E Spurennachweis von Albumin durch Analyse von Antigen Antikorperpraezipitaten Clin chim acta 4 127 1959
49 Lohss F and Ott H (in preparation)
50 Bartter F et al (to be published)
51 Bennhold H Über die Vehikelfunktion der Serumeiweisskorper Ergebn inn Med u Kinderh 42 273 1932
52 Goodman D S The interaction of human serum albumin with long-chain fatty acid anions J Am Chem Soc 80 3892 1958
53 Fredrickson D S and Gordon R S Jr Transport of fatty acids Physiol Rev 38 585 1958
54 Bruton O C Agammaglobulinemia Pediatrics 9 722 1952
55 Good R A and Zak S J Disturbances of gamma globulin synthesis as experiments of nature Pediatrics 18 109 1956
56 Gitlin D Gross P A M and Janeway C A The gamma globulins and their clinical significance New England J Med 260 21 1959
57 Lang N Schettler G and Wildhack R Über einen Fall von Agammaglobulinamie und das Verhalten parenteral zugefuhrten radioaktiv markierten Gammaglobulins im Serum Klin Wehnschr 32 856 1954
58 Ott H Agammaglobulinämie Medizinische Nr 33/34 1242 1958
59 Gitlin D Some concepts of plasma protein metabolism A D 1956 Pediatrics 19 657 1957
60 Craig J M Gitlin D and Jewett T C The response of lymph nodes of normal

and congenitally agammaglobulinemic children to antigenic stimulation (abstract) A M A J Dis Child 88 676 1954
61 Good R A and Varco R L Successful homograft of skin in a child with agammaglobulinemia J A M A 157 713 1955
62 Martin C M Waite J B and McCullough N B Antibody protein synthesis by lymph nodes homotransplanted to a hypogammaglobulinemic adult J Clin Invest 36 405 1957
63 Gitlin D Janeway C A Apt L and Craig J M Agammaglobulinemia in Cellular and Humoral Aspects of the Hypersensitive States edited by H S Lawrence Hoeber New York 1959
64 Porter H Congenital agammaglobulinemia a sex linked genetic trait and demonstration of delayed skin sensitivity (abstract) A M A J Dis Child 90 617 1955
65 Smithies O Zone electrophoresis in starch gels group variations in the serum proteins of normal human adults Biochem J 61 629 1955
66 Smithies O and Walker N F Notation for serum protein groups and the genes controlling their inheritance Nature London 178 694 1956
67 Allison A C Genetic control of human haptoglobin synthesis Nature London 183 1312 1959
68 Allison A C Blumberg B S and ap Rees Haptoglobin types in British Spanish Basque and Nigerian African populations Nature London 181 824 1958
69 Smithies O Third allele at the serum β-globulin locus in humans Nature London 181 1203 1958
70 Harris H Robson E B and Siniscalco M β-globulin variants in man Nature London 182 452 1958
71 Fraser C R Harris H and Robson E B A new genetically determined plasma protein in man Lancet 1 1023 1959
72 Beierwaltes W H and Robbins J Familial increase in the thyroxine binding sites in serum alpha globulin J Clin Invest 38 1683 1959
73 Tanaka S and Starr P A euthyroid man without thyroxine-binding globulin J Clin Endocrinol and Metab 19 485 1959
74 Gordon R S Jr Exudative enteropathy Abnormal permeability of the gastrointestinal tract demonstrable with labelled polyvinylpyrrolidone Lancet 1 325 1959
75 Citrin Y Sterling K and Halsted J A The mechanism of hypoproteinemia associated with giant hypertrophy of the gastric mucosa New England J Med 257 906 1957
76 Homburger F and Petermann M L Studies on hypoproteinemia II Familial idiopathic dysproteinemia Blood 4 1085 1949

## Chapter 46

# Acatalasia*

---

*James B Wyngaarden and R Rodney Howell*

Acatalasia is a rare hereditary condition presently recognized in man only among the Japanese [1–5] though also known to occur in certain breeds of dogs [6] and guinea pigs [7] The disorder was first described in 1949 by Takahara and Miyamato Three years earlier Takahara, a Japanese otorhinolaryngologist, noted that the operative field in a maxillary sinus from which he was removing a putrid tumor turned brown black upon the addition of hydrogen peroxide and did not foam He also noted that blood from this patient turned dark brown on contact with peroxide Investigation revealed that her blood lacked catalase and that several of her family members had the same defect

The disorder was initially designated *acatalasemia* but since it is now known that catalase is absent from liver muscle and bone marrow as well as from the blood the designation *acatalasia* [8] is preferred and will be used in this chapter to specify the entity The term *acatalasemia* will be employed descriptively only

## CLINICAL SYNDROME

Thirty eight cases of acatalasia have been recognized in 17 Japanese families [4 5] About one half have exhibited a peculiar and characteristic oral gangrene In these patients gum and mouth infections occurred usually before age 10 and small ulcerations of the gums were observed as early as the second or third year of life In some patients these ulcerations receded but were followed by new lesions which encroached upon the dental alveoli thereby loosening the teeth After the teeth fell out or were extracted healing frequently occurred but in some instances gangrene with sequestration ensued Extensive erosions into the maxillary

* This work was supported in part by grant CY-4142 from the Public Health Service

sinus or the mandible sometimes led to development of putrid granulating tumors Surgical intervention and bone grafting were then required All degrees of ulceration occurred from minor erosions which healed readily to extensive oral gangrene At times ulceration and necrosis involved the tonsil or the tongue Recurrent episodes of gangrene at various sites in the alveoli with intermittent healing have continued for as long as 10 years [3] Except for the oral lesions and fever in the presence of infection physical examinations were negative Leukocytosis was not reported although mild anemia was observed The absence of severe anemia indicates that the synthetic defect is not primarily one of heme elaboration Treatment was directed toward excision of the gangrenous tissue and eradication of oral infection After all teeth were removed and lesions had healed many patients were permanently free of symptoms

*Illustrative Case (from Takahara [1])*

An 11 year-old girl presented with pain in her mouth Three years earlier small ulcers had begun to appear on her gums Some healed spontaneously but they were soon succeeded by others which spread to involve the dental alveoli thus loosening the teeth When these teeth fell out or were removed healing occurred After extraction of a tooth some weeks prior to her admission a sequestrum developed at the site of the tooth Gangrene followed and progressed despite medical treatment The right cheek swelled causing severe pain and a fever of 101 F developed

The patient was a pale girl with greatly swollen and slightly reddened cheeks The gums about her remaining teeth were gangrenous Erosion of the right maxilla extended into the maxillary sinus and nasal cavity and produced a foul odor Aside from a slight fever the physical examination was negative Hemoglobin was 65 per cent of normal red blood count 3 450 000 per cu mm Erythrocyte sedimentation rate was 35 mm per hr Bleeding and clotting times were normal and serologic examination was negative

The right nasal cavity contained a foul friable granulating tumor A biopsy specimen showed only chronic inflammation Following radical removal of the tumor and curettage hydrogen peroxide was applied to the operative site whereupon the cavity in the maxilla and the surface blood immediately turned brownish black Profs or Takahara supposed silver nitrate had been applied by mistake promptly washed the wound with saline solution and applied hydrogen peroxide from a new bottle only to observe a repetition of the color change He then noted that the black color was caused by blood oozing from the wound and that very few bubbles had formed

The postoperative course was complicated by three recurrences of sequestrum formation necessitating curettage but the lesions healed completely in 2 months One year later ulceration recurred at the site of the extracted teeth and despite treatment again progressed to development of gangrene

After a month of therapy which included surgical drainage the disease was checked Nine months later another recurrence with sequestration was successfully treated with penicillin Subsequently the patient s remaining teeth were pulled whenever ulcers appeared in her gums At the age of

15 she was edentulous but fully developed and apparently well Investigations revealed absence of catalase in her erythrocytes and oral mucosa

Further investigations revealed that three of five siblings of the patient also lacked catalase in their blood Two of them had the same oral disease as the patient A further disclosure was that the parents were second cousins

## CATALASE STUDIES

When hydrogen peroxide solution is dropped into normal blood, gas is evolved and the color does not change When hydrogen peroxide was dropped into the blood of the patient described above it blackened immediately and gas did not appear [1] Blood from three of five siblings gave an identical response These observations suggested to Takahara that blood catalase was deficient Using Warburg's manometric method [9, 10], he established that catalase activity was indeed grossly deficient in blood and also in oral tissue obtained from these patients The results of his initial studies on five patients are shown in Table 46-1, they indicate a virtual absence of catalase activity in both types of tissue in these subjects Subsequently, confirmatory results were obtained in studies of blood of additional patients by a permanganate titration method [11 12] The values in all patients suspected of lacking catalase by the qualitative test approached zero when analyzed by quantitative methods Blood peroxidase levels were normal in all patients [1]

When a small quantity of $H_2O_2$ was added to acatalasemic blood in a

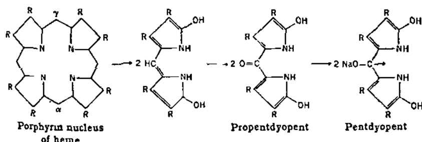
TABLE 46-1 CATALASE ACTIVITY IN BLOOD AND MUCOUS MEMBRANES OF ACATALASEMIC AND NORMAL SUBJECTS

| Case | Sex | Age yr | Material examined | $Q_{O_2}$ Acatalasemic subjects | $Q_{O_2}$ Normal subjects |
|---|---|---|---|---|---|
| 1 | F | 16 | Blood from ear lobe | 7 | 4 500 |
| 2 | M | 18 | Venous blood | 5 | 4 500 |
| | | | Blood from ear lobe | 6 | 4 500 |
| 3 | F | 8 | Blood from ear lobe | 10 | 4 500 |
| | | | Normal oral tissue | 0 | 250 |
| 4 | F | 14 | Blood from ear lobe | 10 | 4 500 |
| 5 | M | 17 | Venous blood | 9 | 4 500 |
| | | | Erythrocytes of venous blood | 8 | 12 000 |
| | | | Normal oral tissue | 0 | 250 |
| | | | Oral ulcer | 0 | |
| | | | Tissue of ethmoidal sinus | 0 | 450 |
| | | | Nasal polyp | 0 | 200 |

$Q_{O_2}$ = cubic millimeters of $O_2$ generated at 38 C in 30 min per mg tissue (dry weight) [8 9]

SOURCE S Takahara [1]

test tube the blood remained black for a long time If an excess of peroxide was added the blood began to lose its dark color in 20 to 30 min and became light reddish brown then lemon yellow and finally colorless This solution was shown to contain propentdyopent a colorless decomposition product of hemoglobin When this solution was made alkaline with potassium hydroxide heated and reduced with sodium dithionite ($Na_2S_2O_4$) a beautiful rose color developed [1] This rose colored compound previously named *pentdyopent* by Bingold [13 14] because of its absorption maximum at 525 mμ is now known to be a dipyrrole [15] The colorless precursor named *propentdyopent* by Fischer and von Dobeneck [16] is a closely related substance The probable structures of these compounds [17] and their relationship to hemoglobin are shown




Takahara next ingeniously correlated the pentdyopent reaction observed in the test tube with biologic events involving bacteria isolated from the oral cavities of affected patients [1] He prepared blood agar plates with normal and acatalasemic blood and planted hemolytic streptococci or pneumococci type I obtained from an acatalasemic subject on these plates These two organisms were the predominating organisms in the mouth lesions of most of the subjects with acatalasia The colonies grew equally well on both types of agar but on the acatalasemic blood agar wide transparent rings appeared around the colony instead of hemolytic rings growing larger with time These colorless zones showed a positive pentdyopent reaction! This study showed that peroxide was produced by these organisms in quantities sufficient to destroy hemoglobin in the blood agar lacking catalase

Kaziro and associates [8] analyzed selected tissues from an asymptomatic adult patient with healed oral lesions and known acatalasemia They found no catalase activity in biopsy specimens of liver muscle and bone marrow although catalase activity was demonstrated in these tissues from a control subject Although this does not constitute exhaustive proof that all viscera are devoid of catalase in patients with acatalasemia these results and those of Takahara showing absence of catalase in oral mucous membranes of such patients provide strong evidence for this likelihood Kaziro et al interpreted their data as evidence for a

"constitutional acatalasia" and proposed the term *anenzymia catalasia* for this genetic anomaly The simpler term *acatalasia* is here proposed as nosologically preferable to *acatalasemia* and as an etymologically acceptable designation for this genetic anomaly

## CATALASE

Thenard [18] noted in 1818 that both plant and animal tissues degrade hydrogen peroxide a substance he himself had discovered some years earlier Loew [20] in 1901 gave the name 'catalase' to the substance responsible for this degradation In 1923 Warburg [9] suggested that catalase is an iron containing enzyme because it is inhibited by cyanide Evidence that its prosthetic group is hematin was offered in 1930 by Zeile and Hellstrom [21] Catalase was crystallized from beef liver in 1937 by Sumner and Dounce [22] who reported an iron content of about 0 1 per cent and emphasized that the prosthetic group is hemin or a closely related substance

### *The Prosthetic Group*

Catalase activity is widely distributed in mammalian tissue but is most abundant in liver kidney and erythrocytes [23 24] Catalase has been crystallized from these three tissues of a number of species [24 25], including man [26–28] as well as from bacteria [29] Animal catalase is an iron porphyrin protein with a molecular weight of 225 000 to 250 000 containing four porphyrin nuclei and four atoms of iron per molecule (0 09 per cent) [30 31] Several authors [32 33] believe that different catalases exist containing 4 3 2 1 and 0 hematin groups and 0 1, 2 3 and 4 verdohematin groups However Bonnichsen has suggested that the oxidized porphyrin groups are post mortem artifacts or changes occurring during isolation and has reported that human liver [26 27] and erythrocyte [34] catalases have no verdohematin groups In human catalase as in beef liver catalase the porphyrin nucleus is the ferric complex of protoporphyrin IX (corresponding to etioporphyrin isomer type III) (protohemin) [35] it is identical with that found in horse radish peroxidase methemoglobin and metmyoglobin and is similar to that of ferricytochrome C The differences in chemical nature and physiologic role of these iron porphyrin proteins are attributed to differences in the protein moieties and in the character of linkages between protein and prosthetic group The structure and biosynthesis of protoporphyrin IX are discussed in Chap 30

The catabolism of the hemin of catalase is apparently identical with that of hemoglobin and ultimately yields bilirubin [36] The ferriprotoporphyrin of catalase is easily removed from the apoenzyme by acid acetone recombination has not yet been achieved [37] The enzyme gives

spectroscopically characteristic derivatives with cyanide fluoride azide and other anions and with its substrate hydrogen peroxide

*The Apoenzyme*

Theorell and Åkeson [38] determined the nitrogen distribution and basic amino acids of horse liver catalase Bonnichsen [34] compared horse liver and blood catalases and found them to contain identical quantities of histidine arginine lysine glutamic and aspartic acids and amide nitrogen Immunologically there was no difference between them and

TABLE 46-2 AMINO ACID COMPOSITION OF BOVINE LIVER CATALASE

| *Amino acid* | *Gm/100 gm protein* | *Moles/mole of catalase* |
|---|---|---|
| Serine | 3.10 | 67 |
| Glycine | 3.26 | 98 |
| Alanine | 4.27 | 108 |
| Threonine | 3.31 | 63 |
| Valine | 6.14 | 118 |
| Methionine | 2.83 | 43 |
| Cystine | 1.12 | 10 |
| Proline | 4.54 | 89 |
| Leucine | 8.60 | 148 |
| Isoleucine | 3.84 | 66 |
| Phenylalanine | [illegible].64 | 104 |
| Tyrosine | 6.80 | 85 |
| Tryptophan | 3.58 | 40 |
| Aspartic acid | 11.40 | 193 |
| Glutamic acid | 9.[illegible]3 | 149 |
| Lysine | [illegible].88 | 152 |
| Arginine | [illegible].91 | 103 |
| Histidine | 7.70 | 122 |
| Ammonia | [illegible].16 | |
| Heme | [illegible].03 | |
| Total | | 1 748 |

SOURCE From G Schnuchel [39]

in the ultraviolet their absorption coefficients were the same Horse blood catalase differed from human blood catalase immunologically

Recently Schnuchel [39] published a complete amino acid analysis of crystalline bovine liver catalase (Table 46-2) It contains 18 amino acids and 1 748 amino acid residues and more histidine and lysine than were reported for horse liver catalase [34 38] Thus regardless of the interpretation of differences in the porphyrin prosthetic groups it is clear that catalases of different species vary in the composition of the apoenzyme

*Tissue Catalases*

Theorell and associates [40] investigated the origin of liver and blood catalase in guinea pigs using $Fe^{59}$ and $Fe^{55}$ Activity in liver ferritin

initially exceeded that in liver catalase but approached equal labeling at about 10 days and thereafter declined in parallel Blood catalase in contrast showed a slower increase that ran parallel to the hemoglobin iron activity They concluded that identical enzymes were being formed in different organs The short life cycle of liver catalase suggested to Theorell [19] that the verdohematin prosthetic groups previously found in liver catalase [32, 33] may be normal intermediates in the destruction of catalase

Additional evidence for independent origins of heme of liver and erythrocyte catalases is provided by studies of Schmid Figen and Schwartz [41] The administration of allylisopropylacetyl carbamide (Sedormid) to rabbits and rats causes simultaneously a marked and rapid fall in liver catalase activity, a great increase in liver porphyrin concentration and porphyrinuria No significant change occurs in erythrocyte catalase activity in hemoglobin concentration or in activity of liver cytochrome oxidase and succinic dehydrogenase Studies with glycine 2 $C^{14}$ suggested that Sedormid blocks formation of catalase in liver, the porphyrin accumulations in liver and urine were viewed as possible consequences of this block The observation that daily excretion of porphyrins and their pyrrole precursors exceeded the amounts of catalase heme normally found in liver may be a reflection of the extremely rapid turnover of liver catalase It also suggests that catalase or a product of it may normally exert a feed back regulatory effect upon hepatic porphyrin synthesis

Finally levels of catalase activity of liver can be varied experimentally but those of erythrocytes remain constant under a variety of circumstances Among the factors which selectively reduce catalase activity of liver or other viscera are starvation [42] iron deficiency [43] copper deficiency [43] castration (in male rats only) [44] adrenalectomy (in rats of both sexes enhanced by prior castration in the male) [44] endogenous [45] or transplanted [46] tumors nitrogen mustard, carcinogens or hepatotoxins [47] Elevations of liver catalase activity are produced in adrenalectomized rats by cortisone and in castrated male rats by testosterone Both hormones are required for complete restoration of normal activity in castrate adrenalectomized male rats Testosterone increased kidney and liver but not blood catalase activity in normal female mice [44]

In severe human iron-deficiency anemia there is no change in catalase activity per milliliter of erythrocytes although there is a slight decrease per million red cells [48] If it is assumed that catalase and hemoglobin derive heme from a common source [40] these results indicate that catalase synthesizing mechanisms compete successfully for a limited amount of heme under conditions of iron deficiency In iron deficiency in animals catalase activity of heart remains normal despite a decline in activity in other viscera [43]

Only one abnormal value of blood catalase activity was noted in 152 patients with a variety of disorders Similar analyses in diseased animals gave only normal results [49] Blood catalase is said to be elevated in acute malaria and normal in the chronic phase [50]

## CATALASE FUNCTION

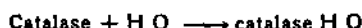
### *Decomposition of $H_2O_2$*

It was originally thought that catalase served as a physiologic safe guard to decompose $H_2O_2$ which might otherwise accumulate within the cell Bingold [51] suggested in 1933 that the role of erythrocyte catalase was to protect hemoglobin from oxidation by $H_2O_2$ It was shown that inhibition of catalase by hydroxylamine permitted rapid oxidation of hemoglobin to methemoglobin when peroxide was added Keilen and Hartree [52] reasoned that the violent decomposition of $H_2O_2$ in Bingold s experiments generated much $O_2$ and maintained hemoglobin as oxy hemoglobin in which form its iron is resistant to oxidation They demon strated formation of methemoglobin despite the presence of abundant catalase activity when $H_2O_2$ was generated slowly by a glucose oxidase system Since this experiment simulated conditions in vivo they con cluded that catalase does not function to protect hemoglobin

### *Peroxidatic Activity*

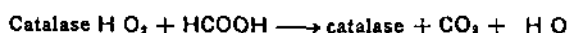
In 1936 Keilen and Hartree [53] demonstrated coupled oxidation of ethanol in the presence of a peroxide generating system (xanthine oxidase and hypoxanthine) and catalase It is now well established that with high substrate concentrations of low molecular weight alcohols formalde hyde or formic acid and low concentrations of peroxide catalase exhibits peroxidatic activity

Chance [54 55] has studied the mechanisms of the actions of catalase The initial reaction involves formation of a complex between catalase and $H_2O_2$ in which peroxide is bound to the ferric iron of heme



$$\text{Catalase} + H_2O_2 \longrightarrow \text{catalase } H_2O_2$$

The enzyme substrate complex can then react with a hydrogen donor —a peroxidatic reaction



$$\text{Catalase } H_2O_2 + HCOOH \longrightarrow \text{catalase} + CO_2 + 2H_2O$$

*Or it can react with a second molecule of $H_2O_2$ —a catalatic reaction*



$$\text{Catalase } H_2O_2 + H_2O_2 \longrightarrow \text{catalase} + O_2 + 2H_2O$$

The catalase-hydrogen peroxide complex has been identified spectro photometrically in aerobically respiring cells of *Micrococcus lysodeikticus* The steady state intracellular hydrogen peroxide concentration in these organisms was calculated to be $10^{-8}$ *M* [57 58]

*Physiologic Roles*

Chance believes that catalase acts both catalatically and peroxidatically in vivo [56] and has pointed out [57] that the partition between catalatic and peroxidatic utilization of hydrogen peroxide will depend mainly upon the steady state peroxide concentration The velocity of the catalatic reaction increases linearly with the peroxide concentration the peroxidatic reaction is essentially independent of peroxide concentration Catalase may thus act as a regulator of hydrogen peroxide concentration, small amounts of peroxide will saturate the peroxidatic function, larger amounts are decomposed in the catalatic reaction The *peroxidatic action* of catalase requires only very low concentrations of peroxide, about $10^{-9}$ *M* Thus at a steady state concentration of $10^{-8}$ *M*, as in *M lysodeikticus* [55, 56], catalase is probably acting by a combination of the catalatic and peroxidatic pathways

Since physiologically there may exist high concentrations of other acceptors and low concentrations of peroxide, it is conceivable that catalase serves primarily as a peroxidase in animal tissues and only secondarily as a defense against accumulation of peroxide Indeed the catalatic reaction $2H_2O_2 \longrightarrow 2H_2O + O_2$ may be viewed as merely a special case of a peroxidatic reaction where hydrogen peroxide serves both as substrate and as acceptor Theorell [19] has proposed that both catalatic and peroxidatic enzymes be called *hydroperoxidases, to emphasize that they have a common substrate* $H_2O_2$

Catalase is not absolutely specific and will act upon organic peroxides such as ethyl hydrogen peroxide under certain circumstances [58 59] Its rate of decomposition of hydrogen peroxide is the highest of any enzymatic rate studied to date One molecule of catalase is capable of splitting 42 000 molecules of $H_2O_2$ per second at 0°C

Catalase is inactivated in 10 min at 60°C but is stable at 4° It is inhibited by a large number of compounds some of which do not affect other heme protein enzymes In hemolyzates of normal blood, inclusion of 2 4-dichlorophenol to inhibit catalase permits demonstration of the peroxidatic action of methemoglobin [60] which will then result in rapid oxidation of uric acid (or other suitable acceptor) in the presence of peroxide Hematin split from catalase by acid acetone will function peroxidatically but not catalatically in this system demonstrating the essential contribution of the catalase protein to its catalatic activity [61]

## HYDROGEN PEROXIDE

The sources of hydrogen peroxide become important in consideration of the pathogenesis of the oral lesions of subjects with acatalasia $H_2O_2$ is produced by aerobic dehydrogenases enzymes which transfer hydrogen

directly to molecular oxygen of which several are found in mammalian tissue All the enzymes of this series employ flavin adenine dinucleotide (FAD) as coenzyme, and occur as cytoplasmic constituents A number have also been identified in the insoluble particles of liver presumably the mitochondria Peroxide formation then must be a normal occurrence in mammalian cells

The important aerobic dehydrogenases of mammalian tissue are monamine oxidase diamine oxidase D amino acid oxidase, L-amino acid oxidase glycine oxidase glycolic oxidase xanthine oxidase and uricase although the last enzyme does not occur in human tissue

That coupled peroxidatic reactions of the types discovered by Thurlow [62] and by Keilen and Hartree [52] may be of physiologic significance was shown by Heppel and Porterfield [63] who found sufficient hydrogen peroxide produced in rat liver homogenates to promote a readily measurable oxidation of nitrite to nitrate by catalase

Aerobic dehydrogenases occur in bacteria also and production of $H_2O_2$ has been detected in several aerobic organisms which contain no catalase such as pneumococci *Bacillus acidophilus* and certain streptococci [51] $H_2O_2$ is presumed to occur in other aerobic or faculative anaerobic organisms also but to escape accumulation because of the presence of catalase which is known to exist in *Escherichia coli Bacillus subtilis Staphylococcus aureus Pseudomonas aeruginosa* and others [51]

## PATHOGENESIS OF LESIONS OF ACATALASIA

Individuals with acatalasia show no signs of ill health other than mouth lesions When all teeth are lost or removed and the lesions treated healing occurs and the individuals remain well thereafter Some subjects with acatalasia are asymptomatic throughout their lives These observations strongly imply that oral sepsis initiates the ulcerative gangrenous process and that individuals with acatalasia are vulnerable to attack by ordinarily innocuous bacteria of normal oral flora

### *Local Lesions*

Takahara [1] reasoned that gums are apt to be injured during mastication and that wounds in gums are suitable media for bacteria The most commonly cultured organisms hemolytic streptococci and pneumococci type I are producers of hydrogen peroxide and lack catalase In persons who lack catalase in blood and oral tissue serious effects result because hydrogen peroxide oxidizes blood reaching the lesion thus depriving the infected area of oxygen and causing necrosis This would favor multiplication of bacteria increased production of peroxide and oxidation of hemoglobin

Additional factors may be involved [8] Several other potential effects

of peroxide on tissue should be considered, such as possible actions upon sessile heme proteins Activity of catalase has been observed to decline in high concentrations of peroxide in the presence of a reducing substance, probably because of autocatalysis of oxidation of the protohematin groups to bile pigment hematin groups [33] In the absence of catalase similar changes might well involve tissue peroxidases, cytochromes, and myoglobin Production of metmyoglobin would render it incapable of binding $O_2$ in muscle Recently, even higher valence states of iron (ferryl myoglobin) [64], or production of free radicles of myoglobin through loss of hydrogen from the conjugated ring system of the porphyrin moiety [65] have been attributed to hydrogen peroxide

Dixon [66] showed that catalase protects xanthine oxidase from destruction by the hydrogen peroxide which it forms This may be an example of the well known susceptibility of flavin prosthetic groups to destruction by peroxide or it may illustrate a general effect of peroxide on proteins Peroxide will oxidize sulfhydryl groups potentially all the way to sulfonic acid residues, and a large percentage of enzymes depend upon sulfhydryl groups for functional activity

Peroxide is hemolytic [67] It is also known to oxidize directly a number of compounds found in cells, e g , α keto acids Thus a large number of effects in addition to destruction of hemoglobin may be anticipated if tissue lacking catalase is exposed to hydrogen peroxide

*Systemic Effects*

About one half of the subjects with acatalasia are asymptomatic, and many of the others remain entirely well when mouth lesions have healed Initially it was not known that tissue catalase was also absent and it remained possible that subjects with acatalasemia lacked catalase only in blood and in injured oral mucous membranes Had this been so, these subjects would have had a catalase distribution like that of the duck, in which blood lacks catalase but other tissues have abundant activity of this enzyme [8] With the demonstration of absence of measurable catalase activity of other tissues in an asymptomatic subject with acatalasemia it became clear that catalase was not an indispensable enzyme and that in its absence toxic levels of $H_2O_2$ did not accumulate Thus an experiment of nature has answered one important question and it is now clear that catalase does not serve as an essential physiologic safeguard One might wonder whether subjects with acatalasia would show increased levels of methemoglobin in circulating erythrocytes but in one analysis such an increase was thought questionable [8]

Recently it has been shown that peroxidases are widely distributed in mammalian tissue [68] and presumably these enzymes utilize such peroxide as is produced as substrate for their oxidative functions It will be recalled that peroxidase was normal in acatalasemic blood pre

sumably representing peroxidatic activity of leukocytes [69 70] Consumption of peroxide in such reactions apparently is quantitatively sufficient to prevent peroxide toxicity even in the absence of catalase unless infection with catalase free organisms supervenes

## TREATMENT

Treatment of oral lesions has been highly successful in preserving life and in eventually controlling the progression of the necrotic changes Surgical excision of granulating tumors *curettage drainage and irrigation* of septic areas extraction of teeth and antibiotic therapy have been employed Reconstructive surgery and bone grafts have been required *The direct application of crystalline* catalase suspensions for control of acute lesions has been suggested [71] The effects of transfusions of normal whole blood containing catalase would also seem worthy of evaluation

## GENETICS

The 38 known cases of acatalasia occurred in 17 families living in various parts of Japan In all but 3 families 2 or more siblings were affected In 2 families as many as 4 siblings were affected A history of consanguineous marriage was obtained from 16 families Of the 38 cases 19 occurred in males and 19 in females The first 5 pedigrees discovered containing 13 cases of acatalasia have been reported in detail [1, 2] *In each one (or more) consanguineous marriage was found a total of 9* in all In 3 families both parents of children with acatalasia were living and were studied Not a single parent had acatalasemia Also in 1 family 12 of 15 children of 3 acatalasemic members were tested and none had acatalasemia These findings virtually exclude dominant inheritance

The findings of consanguinity involvement of families at one sibling level only and equal sex distribution are suggestive of non sex limited recessive inheritance In a study of 27 of 31 members of 5 sibships including known acatalasia subjects 13 acatalasemic and 14 normal members were found [1 2] If one excludes from analysis the 5 propositi the finding of 8 acatalasemic subjects among 22 siblings is in satisfactory agreement with an anticipated incidence of 25 per cent homozygous abnormal individuals from a nonlimited recessive inheritance

Catalase assays have been done on the blood of 66 members of 5 families [72] by a modification of the method of von Euler and Josephson [73 74] The distribution of values of activity units was clearly trimodal (Fig 46 1) In 10 subjects values were zero in 30 they ranged from 1 48 to 2 89 in the remainder they were comparable to values in 206 control subjects which ranged from 3 91 to 7 10 Thus subjects exist in these

families who show intermediate values of catalase activity which do not overlap with values for the controls Presumably these are 'carriers' heterozygous for the acatalasia trait This investigation showed that (1) the parents of acatalasemic subjects are hypocatalasemic, (2) the

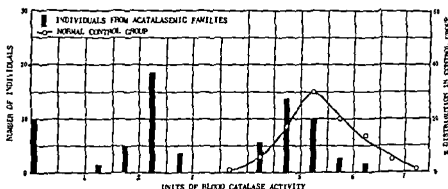



Fig 46-1 Distribution of catalase values for members of five acatalasia families and comparison with a percentage distribution curve of values from a normal control group (*By permission of E T Nishimura et al* and *Science* [2])

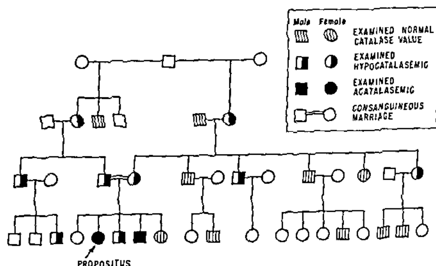



Fig 46-2 Genealogic relationships in an acatalasia family (*Redrawn from E T Nishimura et al* [2] *Reproduced with permission of authors and publisher*)

siblings of acatalasemic individuals may be acatalasemic hypocatalasemic or normal (3) an acatalasemic parent has hypocatalasemic children when mated to a normal person These findings and those of an expanded study recently published [7b] appear to document fully the earlier conclusions of Takahara and coworkers [2 b] that acatalasia is transmitted by incompletely recessive monogenetic inheritance A typical genealogic diagram appears in Fig 46 2

Allison Rees and Burn [6] noted variations in blood catalase activity in a large group of dogs of various breeds In some dogs activity values were quite low and blood darkened on the addition of $H_2O_2$ Controlled breeding of these animals disclosed a simple mode of recessive inheritance for which a single pair of alleles was proposed Heterozygous dogs showed values of blood catalase intermediate between those of homozygous animals and those of normal dogs Tissue catalase values were not reported

Radev [7] obtained similar genetic results in a study of guinea pigs with normal and low blood catalase activity values Animals with low blood values were also found to have low catalase activity values in their livers No signs of ill health were noted in either guinea pigs or dogs with low catalase activities

## SUMMARY

1 Acatalasia is a rare hereditary disorder presently recognized only among the Japanese in which ulcerating gangrenous lesions of the oral cavity may be observed The oral mucous membranes erythrocytes liver, muscle and bone marrow of affected individuals lack catalase activity

2 The lesions appear to be caused by the destructive effects of hydrogen peroxide generated by catalase free bacteria growing within the mouth In subjects with acatalasia hydrogen peroxide injures oral tissue possibly by destruction of structural and enzymatic proteins through oxidation of their sulfhydryl groups by oxidation of heme proteins such as hemoglobin myoglobin peroxidases and the cytochromes and by oxidation of flavoproteins

3 Acatalasia may be entirely asymptomatic and affected persons whose teeth are removed and whose lesions have healed may thereafter be entirely well Catalase appears to be a nonessential enzyme except for the protection it affords against $H_2O_2$ generated by bacteria

4 Acatalasia is transmitted as an autosomal recessive Mendelian characteristic Consanguinity has been found in most pedigrees of acatalasia The distribution of blood catalase activity values in 66 members of 5 families was clearly trimodal and no overlap was observed The intermediary values presumably defined individuals who were heterozygous for the acatalasia trait Both parents some siblings and all children of acatalasemic subjects were heterozygous by this criterion

5 An apparently analogous disorder of catalase synthesis with similar genetic characteristics has been described in certain breeds of dogs and guinea pigs Heterozygous dogs show blood catalase activity values intermediate between those of normal and homozygous animals Guinea pigs with low blood catalase activity values also show low activity in liver

# BIBLIOGRAPHY


1 Takahara S Progressive oral gangrene probably due to lack of catalase in the blood (acatalasaemia) Report of nine cases Lancet 2 1101 1952
2 Takahara S Sato H Doi M and Mihara S Acatalasemia III On the heredity of acatalasemia Proc Japan Acad 28 585 1952
3 Takahara S Progressive oral gangrene due to acatalasemia Laryngoscope 64 685 1954
4 Takahara S and Doi K Statistical observation on 35 cases of acatalasemia appearing in literature Jap J Otol Tokyo 61 1727 1958
5 Takahara S and Doi K Statistical study of acatalasemia (a review of thirty eight cases appearing in the literature) Acta med okayama 13 No 1 1959
6 Allison A C Rees W and Burn G I Genetically controlled differences in catalase activity of dog erythrocytes Nature London 180 649 1957
7 Radev T *Hypoenzymia Catalasea beim Meerschweinchen und deren Vererbung* p 190 Internat Congr Biochem Wien Sept 1–6 1958
8 Kaziro K Kikuchi G Nakamura H and Yoshiya M Die Frage nach der physiologischen Funktion der Katalase im menschlichen Organismus Notiz über die Entdeckung einer Konstitutionsanomalie Anenzymia catalasea Chem Ber 85 886 1952
9 Warburg O Über die antikatalytische Wirkung der Blausaure Biochem Ztschr 136 266 1923
10 Fujita A and Kodama T Manometrische Bestimmung der Katalase Biochem Ztschr 232 20 1931
11 Stern L Die Beziehung des Katalasesystems zu den Oxydationsvorgangen in den Tiergeweben Biochem Ztschr 182 139 1927
12 Stern K O Über die Katalase farbloser Blutzellen Ztschr physiol Chem 204 259 1932
13 Bingold K Weitere Untersuchungen zur Formulierung eines biologischchemischen Blutkreislaufes Klin Wchnschr 14 1289 1935
14 Bingold K Über die Bedeutung von Katalase und Hydroperoxyd fur den Blutstoffwechsel Deutsches Arch klin Med 177 230 1935
15 Fischer H and Muller A Über die Lentdyopent Reaktion I Mitteilung Ztschr physiol Chem 246 43 1937
16 Fischer H and von Dobeneck H F Über die Lentodyopent Reaktion II Mitteilung Ztschr physiol Chem 263 125 1937
17 Lemberg R and Legge J W *Hematin Compounds and Bile Pigments Their Constitution Metabolism and Function* p 417 Interscience New York 1949
18 Thénard L J L Academie des sciences Paris (1818) cited by Theorell [19]
19 Theorell H The iron-containing enzymes B Catalases and peroxidases Hydroperoxidases in *The Enzymes Chemistry and Mechanism of Action* edited by J B Summer and K Myrback vol 2 part 1 p 397 Academic Press Inc New York 1951
20 Loew O Catalase new enzyme of general occurrence with special reference to the tobacco plant U S Department of Agriculture Report 68 1901 p 47
21 Zeile K and Hellstrom H Über die aktive Gruppe der Leberkatalase Ztschr physiol Chem 192 171 1930
22 Sumner J B and Dounce A L Crystalline catalase J Biol Chem 121 417 1937
23 Greenstein J P and Thompson J W Range in activity of several enzymes in normal and neoplastic tissues of mice J Nat Cancer Inst 4 275 1943–1944
24 Sumner J B and Somers G F *Chemistry and Methods of Enzymes* p 216 Academic Press Inc New York 1953
25 Dixon M and Webb E C *Enzymes* Academic Press Inc New York 1958
26 Bonnichsen R K Catalase from horse kidney and human liver Acta chem scandinav 1 114 1947

27 Bonnichsen R K On the hematin and the bile pigments in catalase Acta chem scandinav 2 561 1948
28 Herbert D and Pinset J Crystalline human erythrocyte catalase Biochem J 43 203 1948
29 Herbert D and Pinset J Crystalline bacterial catalase Biochem J 43 193 1948
30 Chance B and Maehly A C Assay of catalases and peroxidases in *Methods in Enzymology* edited by S P Colowick and N O Kaplan vol 2 p 764 Academic Press Inc New York 1955
31 Bonnichsen R Blood catalase in *Methods in Enzymology* edited by S P Colowick and N O Kaplan vol 2 p 781 Academic Press Inc New York 1955
32 Sumner J B Dounce A L and Frampton V L Catalase III J Biol Chem 136 343 1941
33 Lemberg R and Legge J W Liver catalase Biochem J 37 117 1943
34 Bonnichsen R K A comparative study of the blood and liver catalases from the horse Arch Biochem 12 83 1947
35 Stern K G The constitution of the prosthetic group of catalase J Biol Chem 112 661 1936
36 Lemberg R Norris M and Legge J W Catalase Nature London 144 551 1939
37 Sumner J B and Dounce A L Catalase II J Biol Chem 127 439 1939
38 Theorell H and Åkeson Å Nitrogen distribution and basic amino acids in horse radish peroxidase and horse liver catalase determined by a new micro method Ark Kemi Mineral Geol 16A No 8 1943
39 Schnuchel G Untersuchungen an Rinderleber Katalase II Mitteil die Amino sauren des Proteins Ztschr physiol Chem 303 31 1956
40 Theorell H Beznak H Bonnichsen R Paul K G and Åkeson Å On the distribution of radioactive iron in guinea pigs and its rate of appearance in some hemoproteins and ferritins Acta chem scandinav 5 445 1951
41 Schmid R Figen J F and Schwartz S Experimental porphyria IV Studies of liver catalase and other heme enzymes in Sedormid porphyria J Biol Chem 217 263 1955
42 Miller L L Changes in rat liver enzyme activity with inanition Fed Proc 6 2 9 1947
43 Schultze M O and Kuiken K A The effect of deficiencies in copper and iron on the catalase activity of rat tissues J Biol Chem 137 727 1940
44 Adams D H Hormonal factors influencing catalase activity in mice testicular and adrenal factors Biochem J 50 486 1952
45 Greenstein J P Jenette W V and White J The liver catalase activity of tumor bearing rats and the effect of extirpation of the tumor J Biol Chem 141 327 1941
46 Adams D H The mechanisms of liver catalase depressing action of tumors in mice Brit J Cancer 4 183 1950
47 Adams D H and Roe F J C The action of some chemical substances on mouse liver catalase in vivo Brit J Cancer 7 09 1953
48 Beutler L and Blaisdell R K Iron enzymes in iron deficiency II Catalase in human erythrocytes J Clin Invest 37 833 1958
49 Richardson M Huddleson F Trustdorf M and Bethea R Study of catalase in erythrocytes and bacteria III Catalase activity of erythrocytes from humans and animals in various pathological states Arch Biochem & Biophys 47 338 1953
50 Fortunato A and de Lorenzo F Folia med (Naples) 29 350 1943 cited by Richardson et al [49]
51 Bingold K Die Niere als blutzerstorendes Organ Klin Wchnschr 12 1501 1933
52 Keilen D and Hartree E F Properties of catalase catalysis of coupled oxidation of alcohols Biochem J 39 293 1945
53 Keilin D and Hartree E F Coupled oxidation of alcohol Proc Roy Soc London s B 119 141 1936

# BIBLIOGRAPHY


1 *Takahara S* Progressive oral gangrene probably due to lack of catalase in the blood (acatalasaemia) Report of nine cases Lancet **2** 1101 1952
2 Takahara S Sato H Doi M and Mihara S Acatalasemia III On the heredity of acatalasemia Proc Japan Acad **28** 585 1952
3 Takahara S Progressive oral gangrene due to acatalasemia Laryngoscope **64** 685 1954
4 Takahara S and Doi K Statistical observation on 35 cases of acatalasemia appearing in literature Jap J Otol Tokyo **61** 1727 1958
5 Takahara S and Doi K Statistical study of acatalasemia (a review of thirty eight cases appearing in the literature) Acta med okayama **13** No 1 1959
6 Allison A C Rees W and Burn G I Genetically controlled differences in catalase activity of dog erythrocytes Nature London **180** 649 1957
7 Radev T *Hypoenzymia Catalasea beim Meerschweinchen und deren Vererbung* p 190 Internat Congr Biochem Wien Sept 1–6 1958
8 Kaziro K Kikuchi G Nakamura H and Yoshiya M Die Frage nach der physiologischen Funktion der Katalase im menschlichen Organismus Notiz über die Entdeckung einer Konstitutionsanomalie Anenzymia catalasea Chem Ber **85** 886 1952
9 Warburg O Über die antikatalytische Wirkung der Blausaure Biochem Ztschr **136** 266 1923
10 Fujita A and Kodama T Manometrische Bestimmung der Katalase Biochem Ztschr **232** 20 1931
11 Stern L Die Beziehung des Katalasesystems zu den Oxydationsvorgangen in den Tiergeweben Biochem Ztschr **182** 139 1927
12 Stern K O Über die Katalase farbloser Blutzellen Ztschr physiol Chem **204** 259 1932
13 Bingold K Weitere Untersuchungen zur Formulierung eines biologischchemischen Blutkreislaufes Klin Wchnschr **14** 1289 1935
14 Bingold K Über die Bedeutung von Katalase und Hydroperoxyd fur den Blutstoffwechsel Deutsches Arch klin Med **177** 230 1935
15 Fischer H and Muller A Über die Lentdyopent-Reaktion I Mitteilung Ztschr physiol Chem **246** 43 1937
16 Fischer H and von Dobeneck H F Über die Lentodyopent Reaktion II Mitteilung Ztschr physiol Chem **263** 125 1937
17 Lemberg R and Legge J W *Hematin Compounds and Bile Pigments Their Constitution Metabolism and Function* p 477 Interscience New York 1949
18 Thénard L J L Academie des sciences Paris (1818) cited by Theorell [19]
19 Theorell H The iron-containing enzymes B Catalases and peroxidases Hydroperoxidases in *The Enzymes Chemistry and Mechanism of Action* edited by J B Summer and K Myrback vol 2 part 1 p 397 Academic Press Inc New York 1951
20 Loew O Catalase new enzyme of general occurrence with special reference to the tobacco plant U S Department of Agriculture Report 68 1901 p 47
21 Zeile K and Hellstrom H Über die aktive Gruppe der Leberkatalase Ztschr physiol Chem **192** 171 1930
22 Sumner J B and Dounce A L Crystalline catalase J Biol Chem **121** 417 1937
23 Greenstein J P and Thompson J W Range in activity of several enzymes in normal and neoplastic tissues of mice J Nat Cancer Inst **4** 275 1943–1944
24 Sumner J B and Somers G F *Chemistry and Methods of Enzymes* p 216 Academic Press Inc New York 1953
25 Dixon M and Webb E C *Enzymes* Academic Press Inc New York 1958
26 Bonnichsen R K Catalase from horse kidney and human liver Acta chem scandinav **1** 114 1947

# Appendix

# Methods of Coding and Filing Family Records

*Grace J Yesley*

The student of human genetics who is confronted for the first time with an extended pedigree or a collection of moderate sized pedigrees rapidly acquires a healthy respect for the problems inherent in ordering such data Over a period of 15 years the Department of Human Genetics of the University of Michigan has developed a system of recording and coding pedigree data that has proved of convenience in the study of hereditary disease This appendix constitutes the first description of that system The system is composed of three principal sections (1) a numerically arranged *Kindred Folder File* (2) a *Surname Card File* and (3) a *Diagnosis Catalogue*

## KINDRED FOLDER FILE

By placing all information on a kindred (pedigree results of any examinations performed etc ) in a folder each kindred record may be easily separated from the records of other kindreds As the number of kindreds on file accumulates it becomes advantageous to develop an efficient filing system so that (1) records of all kindreds which have a person or persons involved with some particular disease may be quickly taken from the files for examination and study and (2) it may be rapidly ascertained if a person or his family has already been seen at the Clinic

By assigning a kindred number to each kindred the records may be filed numerically Advantages of this system over an alphabetic system of filing are apparent to anyone who has worked with an alphabetic system and been faced with the problem of frequently having to shift almost all the records to make room for those new records which are continually being placed within the system

54 Chance B The primary and secondary compounds of catalase and methyl or ethyl hydrogen peroxide IV Reactions with hydrogen peroxide J Biol Chem 180 947 1949
55 Chance B Enzyme mechanisms in living cells in *The Mechanisms of Enzyme Action* edited by W D McElroy and B Glass p 399 Johns Hopkins Press Baltimore 1954
56 Chance B The state of catalase in the respiring bacterial cell Science 116 ?0 1952
57 Chance B Enzyme substrate compounds Advances Enzymol 12 153 1951
58 Stern K G On the mechanisms of enzyme action A study of the decomposition of monoethyl hydrogen peroxide by catalase and of an intermediate enzyme substrate compound J Biol Chem 114 473 1936
59 Chance B and Herbert D The enzyme-substrate compounds of bacterial catalase and peroxides Biochem J 46 402 1950
60 Keilin D and Hartree E F Purification of horseradish peroxidase and comparison of its properties with those of catalase and methaemoglobin Biochem J 49 88 1951
61 Howell R R and Wyngaarden J B On the mechanism of peroxidation of uric acids by hemoproteins J Biol Chem In press
62 Thurlow S Studies on xanthine oxidase IV Relation of xanthine oxidase and similar oxidising system to Bach s oxygenase Biochem J 19 175 1925
63 Heppel L A and Porterfield V T Metabolism of inorganic nitrite and nitrate esters I The coupled oxidation of nitrite by peroxide forming systems and catalase J Biol Chem 178 549 1949
64 George P and Irvine D H A possible structure for the higher oxidation state of metmyoglobin Biochem J 60 596 1955
65 Gibson J F and Ingram D J E Location of free electrons in porphin ring complexes Nature London 178 871 1956
66 Dixon M Studies on xanthine oxidase V The function of catalase Biochem J 19 507 1925
67 Nitowsky H M and Tildon J T Some studies of tocopherol deficiency in infants and children III Relation of blood catalase activity and other factors to hemolysis of erythrocytes in hydrogen peroxide Am J Clin Nutrition 4 397 1956
68 Neufield H A Levay A N Lucas F V Martin A P and Stotz E Peroxidase and cytochrome oxidase in rat tissue J Biol Chem 233 209 1958
69 Agner K Verdoperoxidase a ferment isolated from leucocytes Acta physiol scandinav 2 (Suppl 8) 1 1941
70 Agner K Crystalline myeloperoxidase Acta chem scandinav 12 89 1958
71 Annotations Blood catalase and oral ulceration Lancet 2 1121 195?
72 Nishimura E T Hamilton H B Kobara T Y Takahara S Ogura Y and Doi K Carrier state in human acatalasemia Science 130 333 1959
73 von Euler H and Josephson K Über Katalase I Ann 452 158 1927
74 Herbert D Catalase from bacteria (*Micrococcus lysodeikticus*) in *Methods in Enzymology* edited by S P Colowick and N O Kaplan vol 2 p 784 Academic Press Inc New York 1955–1957
75 Takahara S Hamilton H B Neel J V Kobara T Y Ogura Y and Nishimura E T Hypocatalasemia a new genetic carrier state J Clin Invest 39 610 1960

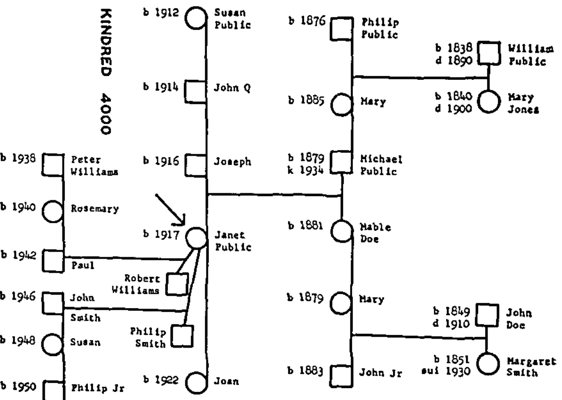
*Pedigree*

A pedigree is the backbone of any study of hereditary disease It may be presented in a number of acceptable forms The conventions employed at the Department of Human Genetics University of Michigan Medical School, have proved to be useful and informative, and are accordingly described here The members of each sibship are recorded in the order of their birth with the eldest on the left and the youngest on the right with a few exceptions which will be discussed later Every pedigree has as a focal point (indicated by an arrow) the propositus, the person who initially brought the kindred to the attention of the physician or geneticist The pedigree then is built around this propositus

The male ancestors of the propositus and his relatives are drawn on the left and the female ancestors and her relatives on the right (Fig 1) To facilitate the drawing and eventual reading of the pedigree an exception is made to the general rule of arranging members of a sibship in their birth order with respect to the parents of the propositus the male parent is drawn at the end of his sibship and the female parent at the beginning of her sibship irrespective of birth order (Fig 1) Each ancestral sibship is also drawn in this manner with the male ancestors at the end of their sibships and the female ancestors at the beginning of their sibships Sibship lettering starts with the sibship of the propositus (Sibship A) and ascends from left to right from that sibship through the sibships of the grandparents Sibship letters A through G then have definite meaning with Sibship B always referring to the father s sibship D to the paternal grandfather s sibship etc (1) The letter H is always reserved for the sibship containing the offspring of the propositus I J K W may be used for any ancestral lines beyond the grandparents which are of special interest

At this stage all sibships descending from the siblings of the ancestors and the siblings of the propositus are identified by the sibship lettering of their parents (the parents biologically related to the propositus) with one letter added That is to say sibships which are first-generation descendants of Sibship B would be labeled BA BB BC etc with Sibship BA being the offspring of the eldest member of Sibship B BB the offspring of the second eldest etc By strict adherence to this coding system in all records one may at a glance determine the exact genetic distance from the propositus of any relative whose sibship lettering starts with a letter from A to H [i e BA BB BC etc = paternal first cousins CA CB CC etc = maternal first cousins BAA BAB BBA etc = paternal first cousins once removed (offspring of paternal first cousins) DAD DBA DBB etc = paternal second cousins etc ]

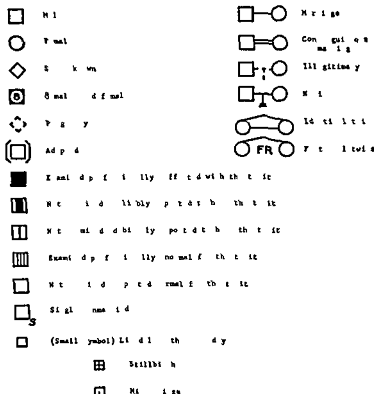
Other occasionally needed and therefore standardized codings are as follows




APPENDIX FIG 1 Hypothetic pedigre illustrating the method used for including names of biologic relatives and spouses birth and death dates

The names of all biologic relatives of the propositus are customarily written above the sibship line and the names of unrelated persons (spouses of relatives) are written below the sibship line Birth and death dates are written below the symbol of each person

Although every individual develops his own method of taking notes during interviews there are in general two approaches to this problem



APPENDIX FIG 3 Symbols frequently used in drawing pedigrees

The family history may be taken in pedigree form all the pertinent information being given on the pedigree or in outline form the data being jotted down by sibship in the sequence in which the pedigree is assembled Figure 3 illustrates the standard shorthand symbols used in drawing pedigrees [1]

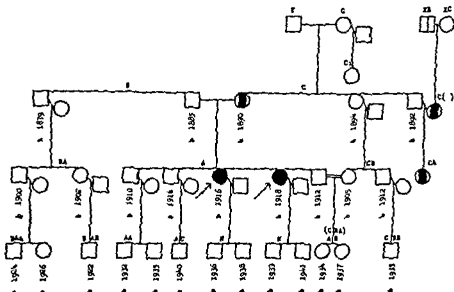
The above steps provide the basic structure of the pedigree coding system with methods for handling the common problems From this point on the problems become more or less specific to the clinic physician or geneticist using the system modifications of or additions to the

[1] Dice Lee R Symbols for human pedigree charts J Heredity 37 11 1946

$_{1}$ $_{2}$ (subscripts) = half siblings ($H_1$ = half siblings of H, $H_2$ = half-siblings of H and $H_1$ i e, children of a third marriage by the parent in common to H and $H_1$)

Y, Z = offspring of person adopted into the family (persons of little or no genetic interest in the study of that particular family but often valuable as informants)

At times one encounters a family in which consanguineous or affinous (relationship between persons through another marriage but not through

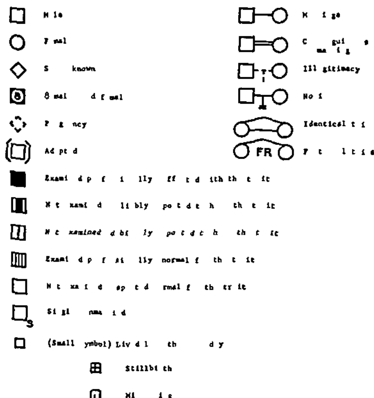


APPENDIX FIG 2 Hypothetic pedigree illustrating sibship lettering system

a blood line ) marriage has occurred Often these relationships may be more readily illustrated if the siblings are drawn out of strict birth order Consanguineous marriages are by convention indicated by a double marriage line Figure 2 illustrates a consanguineous marriage *between a man in Sibship A and a woman in Sibship* CB Here then is another valid exception to following the eldest to youngest rule *established earlier* In the case of consanguineous marriages occurring in a kindred one often has a choice of two possible sibship lettering codes for the offspring Thus offspring from the union referred to above could be designated with either a sibship code of AB (derived from the father s sibship code) or CBA (derived from the mother s sibship code) The code derived from *the parent* who is *more closely* related to the propositus is the one used In Fig 2 the father in the consanguineous union is a sib of the *propositus the mother* is a first cousin—*thus in this case, the* sibship code is derived from the male parent and is therefore AB

The names of all biologic relatives of the propositus are customarily written above the sibship line and the names of unrelated persons (spouses of relatives) are written below the sibship line Birth and death dates are written below the symbol of each person

Although every individual develops his own method of taking notes during interviews there are in general two approaches to this problem



APPENDIX FIG 3 Symbols frequently used in drawing pedigrees

The family history may be taken in pedigree form all the pertinent information being given on the pedigree or in outline form the data being jotted down by sibship in the sequence in which the pedigree is assembled Figure 3 illustrates the standard shorthand symbols used in drawing pedigrees [1]

The above steps provide the basic structure of the pedigree coding system with methods for handling the common problems From this point on the problems become more or less specific to the clinic physician or geneticist using the system modifications of or additions to the

[1] Dice Lee R Symbols for human pedigree charts J Heredity 37 11 1946

system may prove of benefit to meet these specific needs It is convenient to file at least four special forms with the pedigree as follows

1 *Case Record Sheet* The Case Record Sheet is the frontispiece of each kindred folder This sheet contains the following information genetic problem *name of propositus, kindred number origin (how the kindred* came to the attention of the Department), date of ascertainment additional ascertainments and their dates, name of person who conducted the interview and addresses of members in the kindred

2 and 3 *Comment Sheet* and *General Information Sheets* respectively If there is very little family history all the information may be recorded on the Comment Sheet and the General Information Sheets may be *omitted If there is a more extensive family history family history is* recorded by sibship on the General Information Sheets and the Comment Sheet is used by the interviewer to express his opinion concerning the reliability of the information obtained from the informant and the advisability of doing further work with the family

4 *Office Operations Sheet* The Office Operations Sheet is a progress check sheet It contains a list of all the procedures to be carried out *before the kindred folder is ready to be filed*

## SURNAME CARDS

Identification of individuals within kindreds has always presented a complexity of problems Numerous types of name-card files have been set up with varying degrees of success The system developed by the *Department of Human Genetics University of Michigan Medical School* has proved almost 100 per cent effective The file is a ' surname file in which all members of one kindred with the same surname are recorded by sibship on the same 3- by 5 in card *Surname Cards* for the hypothetic kindred shown in Fig 1 would appear as reproduced in Fig 4 The surname is placed in the upper left hand corner and the kindred number in the upper right hand corner By examining the *Public* card and comparing it with the pedigree in Fig 1 it can be seen that each person who uses or has ever used the name ' Public" is recorded on the card (in birth order when more than one individual in a sibship is listed) Within the body of the card the name ' Public ' is abbreviated to P in the cases of married females *and is omitted but understood to be at* the end of the names of all males and all single females Since the propositus is the focal point of the kindred her name is underlined for emphasis If a given name or a surname is unknown a dash may be used in its place Thus —— Wright Phillips would be the name of a woman whose given name is unknown whose maiden name is Wright and married name is Phillips Other examples are Mary —— Phillips (maiden name *unknown*) and Mary Wright —— (married name unknown)

APPENDIX FIG 4 Su name Cards obtained from pe ligree in Fig 1

If a woman bears a child out of wedlock and the father is known this may also be indicated on the Surname Cards For example if an un married woman named Mary Jones bears a child (Tom Jones) whose father is Frank Smith Mary s name would be recorded

Mary Jones (Smith) ( Mrs Frank Smith)

and Tom s name would be recorded

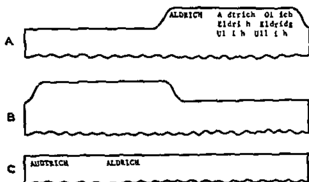
Tom (Smith) Jones

Thus a surname in parentheses is one which was never used by the in dividual but which at the same time has a certain amount of biologic importance, if the unused surname is unknown it may be recorded (——) with the parentheses indicating unused and the dashes indicating un known Quotation marks around a surname indicates that the surname is or has been in use by that individual It may be an assumed name a name acquired when the person was adopted or a maternal surname the use of which is contrary to the custom in this country *Legally* Tom s surname is Jones but the use of the mother s surname is a break from the

tradition of our society, in which the child has the same surname as the father, and is therefore enclosed in quotation marks

Surname Cards are filed in a phonetic alphabetic system combining the surnames which are either the same or almost the same phonetically Thus as an example, cards for the surnames of Allman Ahlman, Alhman Allmond, Alma, Almond, Ammon, Elmen Ullman and Ammons or of Baers, Baer, Baher, Bahers, Bahr, Bahrs, Bair, Bairs Bear, Bears Beer Beers, Bearss, and Behr would be filed as a unit, alphabetically under the most common surname in the group

As soon as one starts filing Surname Cards in a phonetic manner within an essentially alphabetic system, he must devise a system to keep those




APPENDIX FIG 5 A Guide card which precedes phonetically filed Surname Cards—located in file in the alphabetic location of Aldrich B Guide card which follows phonetically filed Surname Cards C Index card which is filed alphabetically in place of Surname Cards with the surname of Audtrich Audtrich Surname Cards have been filed alphabetically under Aldrich

phonetically filed cards together as a unit and at the same time make it very apparent that the cards are out of alphabetic order so they will not be used as guides for filing subsequent cards One half-cut separator guide cards are used to separate that group from the cards filed alphabetically before and after the group The *key name* (ideally the most common name in the group) determines where the group is to be filed it is typed in upper case letters on a card with a second place cut and all other surnames to be filed within this group are typed in lower case on the same card (Fig 5A) A blank first place cut index card is then placed at the end of the group to separate it from the other cards in the file (Fig 5B)

For the names filed out of alphabetic order by being placed in one of these groups 3 by 5 in cards of a color different from the regular Surname Cards are filed in the system where those names would appear if they had been filed in alphabetic order On these cards is typed information which will direct one to the proper place in the file (Fig 5C)

With this system in use the name of *every* individual in *every* kindred

appears in the name file with a minimum use of space By having all relatives with the same surname on one card one can more readily determine whether a newly ascertained person is already on file this is a very important tool in the case of more common surnames Many Mary Smiths may be on file but the number of Mary Smiths with a brother John is much more limited

SUSAN SMITH

469-CAA S S
1555 DB S S Phillip
4000 $H_1$ S S
4938 A S Ma i S

PHILIP SMITH

156-CA P S
2597 B P S
4000 A( ) P S Sr
4000 $H_1$ P S Jr

MARK SMITH

982 D M Antho y S
3376-BA M S
4270 B M S

JOHN SMITH

270 A J R b t S
2254 A( ) J S S
2254 A3 J S Jr
4000-$H_1$ J S
4235 A J S

JANET SMITH

1051 B Mary J S
1732-CB J S R berts
3689 A J S
4000 A J. P bli Willi m S
5104 H J Mari S

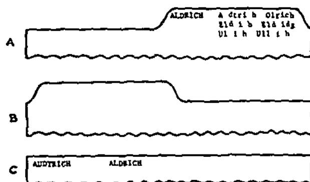
APPENDIX FIG 6 Typical First name Cards

In the files of the Department of Human Genetics University of Michigan Medical School approximately 1 out of every 20 kindreds has at least one person who has or has had the surname of Smith It is apparent that as the files increase in size a further refinement of the system is advantageous in dealing with some of the more common surnames *First name Cards* are typed for the surnames that are common to at least 25 kindreds *Thus all Mary Smiths on record are entered on a Mary Smith* card all John Smiths on a *John Smith* card etc Figure 6 illustrates typical First name Cards These cards are filed alphabetically by given name immediately after the Smith Surname Cards and are separated from the rest of the file by one-fifth cut guide cards of a different color

tradition of our society, in which the child has the same surname as the father, and is therefore enclosed in quotation marks

Surname Cards are filed in a phonetic alphabetic system combining the surnames which are either the same or almost the same phonetically Thus as an example, cards for the surnames of Allman, Ahlman, Alhman Allmond, Alma, Almond, Ammon, Elmen Ullman and Ammons or of Baers, Baer, Baher Bahers, Bahr, Bahrs, Bair, Bairs, Bear, Bears Beer Beers, Bearss, and Behr would be filed as a unit, alphabetically under the most common surname in the group

As soon as one starts filing Surname Cards in a phonetic manner within an essentially alphabetic system he must devise a system to keep those




APPENDIX FIG 5 A Guide card which precedes phonetically filed Surname Cards—located in file in the alphabetic location of Aldrich B Guide card which follows phonetically filed Surname Cards C Index card which is filed alphabetically in place of Surname Cards with the surname of Audtrich Audtrich Surname Cards have been filed alphabetically under Aldrich

phonetically filed cards together as a unit and at the same time make it very apparent that the cards are out of alphabetic order so they will not be used as guides for filing subsequent cards One half cut separator guide cards are used to separate that group from the cards filed alphabetically before and after the group The *key name* (ideally the most common name in the group) determines where the group is to be filed, it is typed in upper case letters on a card with a second place cut and all other surnames to be filed within this group are typed in lower case on the same card (Fig 5A) A blank first place cut index card is then placed at the end of the group to separate it from the other cards in the file (Fig 5B)

For the names filed out of alphabetic order by being placed in one of these groups 3 by 5 in cards of a color different from the regular Surname Cards are filed in the system where those names would appear if they had been filed in alphabetic order On these cards is typed information which will direct one to the proper place in the file (Fig 5C)

With this system in use the name of *every* individual in *every* kindred

# Index

Page numbers in boldface type indicate the main discussion of a subject Page numbers in italic refer to a genetic chart Contributors of chapters are indicated by boldface page numbers

Suppose at some future date a young man named Mark Smith should present himself for examination In an interview with him it is learned that he has siblings named John, Philip and Susan Smith Smith First name Cards for these four names (Mark John, Philip and Susan) are pulled from the files and compared to see if any of the people listed have any *common kindred number and sibship coding On the John, Philip* and Susan cards it is noted that each card lists an individual belonging to Kindred 4 000 who is in Sibship $II_1$ (Fig 6) but there is no Mark Smith listed in Kindred 4 000 $II_1$ However with this amount of positive correlation, the information obtained from Mark and that contained in Kindred 4 000 should be compared—birth dates, other names and ad dresses are all of help in determining whether or not the kindreds are *one and the same In this example there were three positive correlations* However, in attempting to trace relationships it is sometimes helpful to compare records with as few as two positive correlations If Mark had supplied no information about siblings but did state that his mother s name was Janet Public Smith a card in the file with Janet —— Smith (woman with given name of Janet, maiden name unknown married name of Smith) would supply one positive correlation A card in the *file with the name "—— Public Smith (a woman whose given name is* unknown maiden name is Public and married name is Smith) would also supply one positive correlation Thus the location of the name Janet Public Smith in the file (Fig 6) would supply the two positive correlations required for further checking

## DIAGNOSIS CATALOGUE

With the increase in number of records on file a Diagnosis Catalogue becomes a helpful tool for locating all kindred records relating to a particular disease This catalogue could be of loose leaf notebook or card type depending on how much information is to be recorded about each kindred *Each disease is recorded on a separate sheet or card* these are kept in alphabetic order Since many clinical entities may be referred to by more than one name (imperforate anus vs atresia ani) or may be alphabetized under more than one word (*Patellar nail or Nail patellar* syndrome) a list of the synonyms of diseases already on file should be kept so that any disease *apparently* not yet in the *catalogue may be* checked against this list It has proved helpful to have the following *information* on each diagnosis sheet trait kindred number kindred name (surname of the propositus or in the case of a married female maiden surname plus married surname) year of examination type of inheritance number examined and affected with the disease origin and remarks

B

F

## G

**F**

## N

Q



R

## W

## V

W

## V